UNIVERSITY OF EDINBURGH

A Thesis submitted

by

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a candidate to qualify

for the degree

of

DOCTOR OF PHILOSOPHY

May, 1942.

Title:

Constitutional Studies in the Sugar Group.

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Contents

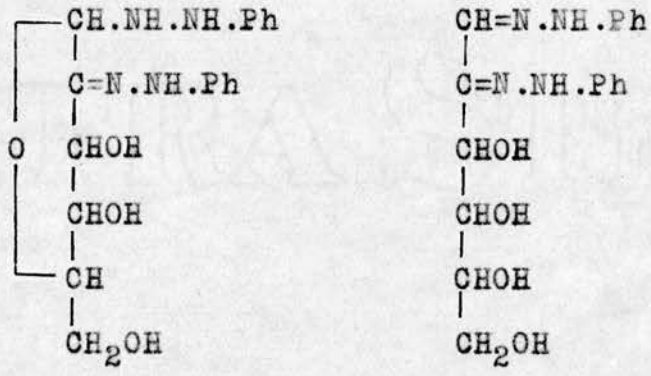
P_A_R_T I

The Structure of Sugar Osazones and Osazone Anhydrides.

The Structure of Sugar Osazones and Osazone Anhydrides

General Introduction

IN his book 'The Constitution of Sugars' (1) W.N. Haworth suggests the possibility that sugar osazones may exist in a cyclic form (I) and not in the acyclic



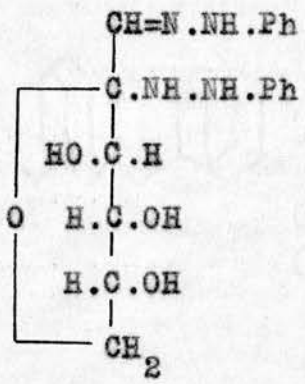
(I) (II)

form (II) proposed by Emil Fischer (2). The only evidence mentioned in favour of this hypothesis was the fact that osazones appear to mutarotate in solution. Failure to characterise in any one case pure α- and β-forms, allied to the probability of decomposition in solution appeared to make it desirable to decide this point by chemical methods. Although such methods only enable us to decide with certainty on the structure of some derivatives of the osazones such/

such as methylated osazones or anhydro-osazones the evidence obtained enables one to suggest that in all the cases examined so far a cyclic structure does indeed exist in the sugar osazones, or that at any rate this represents the most stable structure under the conditions studied. The possibility that the acyclic structure exists in equilibrium with one or more cyclic forms cannot be excluded but osazone derivatives of proved straight chain character have yet to be isolated.

In 1935 the author (3) subjected d-glucose-phenylosazone to methylation. A crystalline monomethyl glucosazone was isolated, m.p. 116-117°, $[\alpha]_D$ -50° in alcohol, different from 3-, 4-, and 6-methyl glucosazones. The monomethyl fructose obtained with difficulty from this product was shown to yield a methylfructopyranoside under conditions favourable to the formation of a furanoside and yielded tetramethyl fructopyranose on complete methylation. The hydroxyl group on C_5 was therefore occupied by a methoxyl residue and the monomethyl osazone was 5-methyl glucosazone. Subsequently this substance has been prepared from 5-methyl glucose by L. v.Vargha (4) who reported m.p. 128°, $[\alpha]_D$-72.2°$\longrightarrow$ $[\alpha]_D$-64.4° in alcohol. Although there is a considerable difference in the physical constants one may point out that/

that a large number of crystallisations were necessary to purify the monomethyl glucosazone prepared by methylation so that a trace of impurity may have persisted. An alternative possibility is that v. Vargha's preparation was stereochemically pure. This being the case it was clear that if glucosazone is cyclic the ring cannot involve C_5. Complete methylation yielded a substance described at the time as a trimethyl glucosazone (3), but recognised later as N-methyl trimethyl glucosazone (5). This was converted to a trimethyl sugar having properties consistent with those of 3:4:5-trimethyl fructopyranose. From this evidence it was concluded that d-glucosazone possesses a ring structure, the ring joining C_2 and C_6 (III)

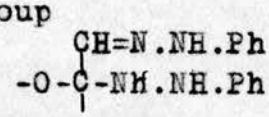


(III)

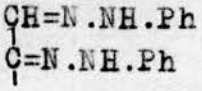
d-Glucosazone is thus formulated as a fructopyranose derivative; this is of interest in connexion with the observation that d-fructose in which the pyranose structure exists (6) yields glucosazone very much more rapidly than d-glucose.

A/

A contrary view was expressed in a publication also in 1935 by L.I.Engel (7). Although this worker carried out methylation experiments with glucosazone he based no conclusions on the results of these owing to his inability to isolate pure crystalline chemical individuals. By methylation by various methods, including the technique of methylation in liquid ammonia suggested by I.Muskat (8), he was unable to reach a methoxyl content greater than 20.7%; this value however approaches that of a trimethyl derivative (22.5% for N-methyl trimethyl glucosazone) and seems to the present author to indicate clearly that an oxide ring exists in the starting material. The main evidence adduced by Engel concerns the similarities between the absorption spectra of glucosazone and its methylated and acetylated derivatives with that of glycerosephenylosazone. Since the latter is presumed to be acyclic the author argues a similar constitution for glucosazone and similar compounds. It would seem however not improbable that the effect on the absorption of the group



may not differ greatly from that of



owing to the presence in both formulae of a doubly bonded nitrogen atom and two aromatic nuclei./

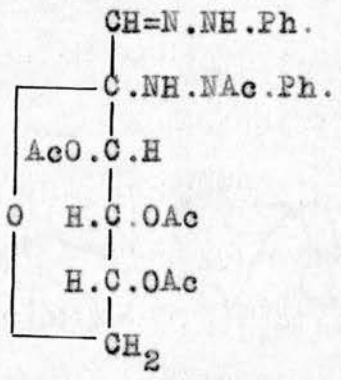
nuclei.

Engel shows clearly that on treatment of fructose phenylmethylosazone with phenylhydrazine in the cold, substitution of the phenylmethylhydrazine residues by phenylhydrazine residues occurs; similarly with p-nitrophenylhydrazine, and by this means seeks to explain the change of rotation of osazones in solution as due to decomposition and not a true mutarotation. This may or may not be the case, but in the view of the present author such exchange reactions could occur equally readily with osazones of the cyclic structure proposed.

Engel (7), Maurer and Schiedt in 1935 (9) and Wolfrom, Konigsberg and Soltzberg (10) in 1936 described a tetra-acetyl glucosazone and the last authors a tetra-acetyl galactosazone. Wolfrom and his co-workers claimed that all the acetyl groups are linked to carbon through oxygen by the application of the method of Kunz and Hudson (11) to distinguish between NAc and OAc groups, and conclude that these osazone acetates are acyclic, although it is pointed out that this does not invalidate the conclusions of E.E.Percival and E.G.V.Percival (3) as to the structure of methylated glucosazone.

These results were denied by E.G.V.Percival (5) who concluded that only three OAc groups were present since only the equivalent of three acetyl groups were removed by deacetylation in the cold whereas four were removed/

at room temperature. Since Behrend and Reinsberg (12) had pointed out that acetylation on the nitrogen of a true hydrazone in the cold is difficult whereas phenylhydrazides readily undergo acetylation under the same conditions it was suggested that the N-acetyl group was to be found on the phenylhydrazide residue (IV).



(IV)

THE METHYLATION OF GLUCOSEPHENYLOSAZONE AND ITS FORMULATION AS A DERIVATIVE OF FRUCTOPYRANOSE

BY

(Mrs.) ELIZABETH E. PERCIVAL

AND

EDMUND G. V. PERCIVAL

Reprinted from the Journal of the Chemical Society,
October, 1935.

Reprinted from the Journal of the Chemical Society, 1935.

335. *The Methylation of Glucosephenylosazone and its Formulation as a Derivative of Fructopyranose.*

By (Mrs.) Elizabeth E. Percival and Edmund G. V. Percival.

According to many workers, osazones such as glucosazone, galactosazone (Levene and Laforge, *J. Biol. Chem.*, 1915, **20**, 429), and 3-methyl glucosazone (Anderson, Charlton, and Haworth, J., 1929, 1329) in solution in alcohol or pyridine exhibit mutarotation which, unless the rotational changes are due to decomposition, may indicate the existence of some type of ring structure.

After a single methylation of glucosephenylosazone with methyl sulphate and sodium hydroxide, excess of alkali being avoided, a new crystalline *monomethyl glucosazone* was isolated, which did not agree in physical properties with any of the known monomethyl glucosazones (see Table I). It was a true osazone, since treatment with *p*-nitrobenzaldehyde gave an osone, from which the original osazone was regenerated in five minutes at room temperature by treatment with phenylhydrazine acetate.

Table I.

Glucosazone.	M. p.	$[\alpha]_D$ in alcohol.	Form.	Reference.
3-Methyl	178—179°	− 109° → − 9°	Needles	Anderson, Charlton, and Haworth, J., 1929, 1329.
4-Methyl	158—159	− 33 → − 15	Needles	Pacsu, *Ber.*, 1925, **58**, 1463; Schinle, *Ber.*, 1932, **65**, 315; Munro and Percival, this vol., p. 873.
6-Methyl	184—187	− 69; no mutarotation	Needles	Helferich and Günther, *Ber.*, 1931, **64**, 1276.
New methyl	116—117	− 50 → − 12	Plates (aqueous alcohol) Needles (equilibrium solution in alcohol)	

The evidence presented below gives no reason to doubt that the new compound is the missing 5-methyl glucosazone.

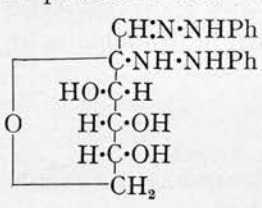
p-Nitrobenzaldehyde, having given a better yield of glucosone from glucosazone than benzaldehyde (Fischer and Armstrong, *Ber.*, 1902, **35**, 3143), was used to prepare the methyl glucosone. The yield was poor (10%) and attempts to improve it by using hydrochloric acid (Fischer, *Ber.*, 1889, **22**, 87) failed. By reduction with zinc dust and acetic acid (Fischer, *loc. cit.*) the corresponding ketose was obtained as a syrup of negative rotation, from which the original osazone could be regenerated in the usual manner. Its properties agreed with those of a monomethyl fructose and the negative rotation indicated its relationship to fructopyranose, so substitution in position 6 was unlikely.

The course of glycoside formation in the cold was followed as described by Levene, Raymond, and Dillon (*J. Biol. Chem.*, 1932, **95**, 699). A comparison of the results (Table III) with those given by fructose (Table II) under parallel conditions showed that, whereas the sugar under review gave 62% of a pyranoside in 24 hours, fructose was exclusively transformed into furanoside. A small constant amount of furanoside appeared to be formed, but this was ascribed to the presence of a 5-methyl aldose produced by a Lobry de Bruyn transformation during the removal of zinc with barium hydroxide. These observations are in harmony with the substitution of methyl in the penultimate hydroxyl group in fructose, such a 5-methyl fructose being capable only of a pyranose formulation. The sugar was accordingly transformed into the pyranoside by heating with methyl-alcoholic hydrogen chloride, and methylation, followed by distillation and hydrolysis, yielded crystalline tetramethyl fructopyranose, indicating that we were dealing with a genuine derivative of fructopyranose.

That 5-methyl glucosazone can be so readily obtained from glucosazone is to be ascribed to the fact that the particular hydroxyl group in question is the most vulnerable to attack by methyl sulphate and alkali. Obviously, whatever rings, if any, exist in the original glucosazone, neither a 1 : 5- nor a 2 : 5-oxide ring is possible.

In order to obtain more evidence on this point glucosazone was methylated by three treatments with methyl sulphate and sodium hydroxide, followed by three with methyl iodide and silver oxide. A red syrup, the methoxyl content of which could not be increased by further methylation, was obtained which gave the analytical figures required for trimethyl glucosazone. Evidently, therefore, there is only one ring in glucosazone, provided that one position is not made unavailable for methylation by steric effects. Repeated attempts to crystallise the syrup failed and it was apparently not identical with the crystalline 3 : 5 : 6-trimethyl glucosazone of Anderson, Charlton, and Haworth (*loc. cit.*) or the 3 : 4 : 6-trimethyl fructosazone of Haworth and Learner (J., 1928, 619). 3 : 4 : 5-Trimethyl fructopyranose has been prepared (Irvine and Patterson, J., 1922, **121**, 2159; for structure see Haworth, Hirst, and Learner, J. 1927, 1040), but there is no record of its phenylosazone.

The syrupy trimethyl glucosazone was converted into a trimethyl fructose by way of the osone as before. Methyl-alcoholic hydrogen chloride reacted slowly at room temperature to form almost exclusively a pyranoside (Table IV), again indicating substitution in position 5 and a free hydroxyl group in position 6. Further methylation of this pyranoside, followed by hydrolysis, again yielded tetramethyl fructopyranose, proving that the sugar was essentially 3 : 4 : 5-trimethyl fructose. Position 6 in the glucosazone is therefore either prevented from undergoing methylation by steric effects, which is improbable, or is concerned with ring formation, and it is considered probable that the osazone contains a pyranose ring and has the annexed structure. Examination of a model of this substance shows that the hydroxyl group on carbon atom 5 is the one most remote from the phenylhydrazine residues, and this may be the reason for the preferential formation of 5-methyl glucosazone.



Although 3-, 4-, and 5-methyl glucosazones may all have the fructopyranose structure, this cannot be the case for 6-methyl glucosazone. The observation of Helferich and Günther (*loc. cit.*) that the purest 6-methyl glucosazone so far obtained exhibits no muta-

rotation in alcohol is in agreement with this, though earlier workers (Kuhn and Ziese, *Ber.*, 1926, **59**, 2314; Ohle and v. Vargha, *Ber.*, 1929, **62**, 2434; Levene and Raymond, *J. Biol. Chem.*, 1932, **97**, 751) had apparently observed mutarotation in this case. The fact that 6-methyl glucosazone is only slowly precipitated (6 hours) during its formation from 6-methyl glucose in the usual way may also have some significance in connexion with its structure.

Experimental.

5-Methyl Glucosazone.—Methyl sulphate (60 c.c.) and 30% sodium hydroxide solution (140 c.c.) were added to glucosazone (20 g.), dissolved in acetone (50 c.c.) and alcohol (125 c.c.), during 2 hours with constant stirring at 50°. The mixture was then maintained at 70° for 10 minutes, diluted with hot water (500 c.c.), neutralised with glacial acetic acid, and kept overnight. The yellow precipitate and brown tarry matter were filtered off and dissolved in boiling alcohol, and water added until precipitation was just maintained on further heating. The tar that separated was removed; the filtrate on cooling deposited a yellow precipitate, which was collected and subjected to further treatment as above. After ten recrystallisations a pale yellow solid was obtained (Found : OMe, 5·0%). This was dissolved in boiling chloroform; unchanged glucosazone crystallised from the cold solution, and the mother-liquor on removal of the solvent under diminished pressure left a product, which was crystallised from aqueous alcohol (Found : OMe, 9·3%). Fractional crystallisation from aqueous alcohol then gave *5-methyl glucosazone* (5 g.) in shining rectangular plates with saw-like edges, m. p. 116—117°, $[\alpha]_D^{20°}$ — 44° in chloroform (*c*, 0·7), — 49° in alcohol (10 mins. after dissolution; *c*, 0·7), — 12° (36 hrs., constant value). This equilibrium solution crystallised in fine needles, m. p. 117° (Found : C, 61·3; H, 6·5; OMe, 7·9; N, 14·8. $C_{19}H_{24}O_4N_4$ requires C, 61·3; H, 6·45; OMe, 8·3; N, 15·0%).

Conversion of Glucosazone into Glucosone.—(1) Glucosazone (1 g.) was dissolved in alcohol (30 c.c.), and water added to produce a turbidity. The vigorously stirred mixture was heated with benzoic acid (0·5 g.) and benzaldehyde (10 c.c.) on a boiling water-bath for 1 hour. After 30 minutes water (50 c.c.) was added. The cooled filtered solution was extracted with ether and evaporated to 20 c.c. at 35° under diminished pressure; on treatment with phenylhydrazine acetate a yellow precipitate of glucosazone appeared after 3 minutes at room temperature (yield, 7%).

(2) Glucosazone (1 g.), in alcohol (100 c.c.), was stirred with benzoic acid (1 g.) and *p*-nitrobenzaldehyde (5 g.) at 90—100° until all the solid had dissolved. Water (150 c.c.) was then added, and the heating continued for 65 minutes, alcohol (50 c.c.) being added after 30 minutes to replace the loss by evaporation. The solution was cooled, filtered (residue A), extracted three times with ether, and evaporated to 20 c.c. at 35° under diminished pressure; treatment with phenylhydrazine acetate gave glucosazone (0·3 g.). The residue (A), similarly treated with *p*-nitrobenzaldehyde (3 g.), yielded glucosazone (0·1 g.), so the conversion was complete to the extent of 40% (cf. Fischer, *loc. cit.*).

Conversion of 5-Methyl Glucosazone into 5-Methyl Glucosone.—By method (2) above, a light yellow syrup of the osone was obtained, which strongly reduced Fehling's solution and on treatment with phenylhydrazine acetate gave a yellow precipitate after 5 minutes in the cold (yield, 10%). On recrystallisation this gave the characteristic shining plates of 5-methyl glucosazone, m. p. 116—117° (Found : OMe, 7·8%).

Reduction of 5-Methyl Glucosone to 5-Methyl Fructose.—5-Methyl glucosazone (5 g.) was converted into 5-methyl glucosone in the above manner. After extraction with ether, in order to avoid decomposition, zinc dust (1 g.) and glacial acetic acid (0·5 c.c.) were added and the solution was evaporated to 80 c.c. This was heated with zinc dust (20 g.) and a few drops of platinic chloride solution on a boiling water-bath with vigorous stirring for 90 minutes during the addition of glacial acetic acid (8 c.c.). A portion (1 c.c.) of the cooled filtered solution failed to give an osazone on treatment with phenylhydrazine acetate in the cold or on heating for 10 minutes, but after 1 hour's heating the osazone came down in the characteristic plates. 2*N*-Barium hydroxide was added to the main bulk until all the zinc was precipitated as zinc hydroxide; the filtrate gave no precipitate with ammonium sulphide. Barium was removed by means of 2*N*-sulphuric acid, and the filtered solution evaporated to dryness at 40°/20 mm. The resulting solid was extracted three times with absolute alcohol (200 c.c.), and the extracts evaporated to dryness, leaving a pale yellow, reducing glass (0·3 g.) consisting of barium acetate mixed with monomethyl fructose. It was considered inadvisable to remove the whole of the

barium because of the danger of decomposition in the presence of a trace of sulphuric acid, but solution in water and addition of more sulphuric acid removed a large part of the inorganic material. The liquid was filtered and evaporated at 40°/20 mm. $[\alpha]_D^{20°}$ — 40° in water (*c*, 0·5) (Found: OMe, 11·8; Ba, 3·8. The assumption that all the barium was present as barium acetate gives $[\alpha]_D^{20°}$ — 50°; OMe, 14·8. Calc. for $C_7H_{14}O_6$: OMe, 16·1%).

Attempted Furanoside Formation.—The method of Levene, Raymond, and Dillon (*loc. cit.*) is by no means quantitative as regards the estimation of fructofuranoside by hydrolysis with 0·1*N*-hydrochloric acid at 100°, since it was found that the monomethyl methylfructopyranoside was hydrolysed to the extent of 15% under the experimental conditions. This is not surprising, since 1 : 3 : 4 : 5-tetramethyl methylfructopyranoside is completely hydrolysed by 0·7*N*-hydrochloric acid during 30 minutes. Further, the effect of acid treatment on the free sugar itself under the conditions obtaining during the hydrolysis is such that a higher reducing value is obtained by the Hagedorn–Jensen ferricyanide method, modified by Hanes (*Biochem. J.*, 1929, **23**, 99), notably to the extent of 25%. The figures recorded for the percentages of pyranoside and furanoside are corrected according to these factors. Table III shows the effect at 20° of 0·5% methyl-alcoholic hydrogen chloride on monomethyl fructose (*ca.* 0·3%). The method employed was to withdraw two samples of 1 c.c. at a time, one being treated with a 20% excess of 0·4*N*-sodium carbonate solution, the volume made up to 5 c.c., 5 c.c. of the standard potassium ferricyanide–sodium carbonate mixture [8·25 g. $K_3Fe(CN)_6$, 10·6 g. Na_2CO_3/litre] added, and the solution heated for 15 minutes at 100°. After cooling for 3 minutes, 5 c.c. of a solution (potassium iodide, 12·5 g., zinc sulphate, 25·0 g., and sodium chloride, 125·0 g./litre) were added, followed by 3 c.c. of 1% acetic acid, the liberated iodine being titrated with 0·015*N*-sodium thiosulphate. The difference between this titre and a blank carried out under the same conditions gave the figure for the reducing value. To the second 1 c.c. portion, 0·4*N*-hydrochloric acid and water were added so that the solution was 0·1*N* with respect to hydrochloric acid, and the solution was heated at 100° for 10 minutes. Sodium carbonate (20% excess) was then added as before, the amounts being adjusted to bring the final volume to 5 c.c. The reducing power was then determined as above. Table II shows parallel experiments with fructose.

TABLE II.

Time.	0·015*N*-Thiosulphate, c.c. Before hydrolysis.	After hydrolysis.	Free sugar, %.	Furanoside, %.	Pyranoside, %.
0	4·2	4·5	100	—	—
40 mins.	0·1	4·5	2	100	—
3 hrs.	—	4·4	—	100	—
5 ,,	0·1	4·6	2	100	—
24 ,,	—	4·5	—	100	—

TABLE III.

Time.	0·015*N*-Thiosulphate, c.c. Before hydrolysis.	After hydrolysis.	Free sugar, %.	Furanoside, %.	Pyranoside, %.
0	5·2	6·5	100	—	—
30 mins.	3·4	5·1	65	14	21
2 hrs.	3·0	4·6	58	14	28
10 ,,	1·8	3·2	35	15	50
24 ,,	1·2	2·5	23	15	62

At the end of 24 hours the solution was still reducing to Fehling's reagent. The table shows that, whereas furanoside formation from fructose is complete after 1 hour, in the case of the sugar under review about 14% of furanoside appears to be formed at once but this figure remains constant while the amount of pyranoside gradually increases. The method is only regarded as semi-quantitative, indicating that in contrast with fructose the sugar forms a pyranoside in preference to a furanoside, an observation which agrees with the structure assigned to the monomethyl osazone.

Preparation of 5-Methyl Methylfructopyranoside and Methylation of the Product.—The sugar (0·25 g.) was dissolved in 3% methyl-alcoholic hydrogen chloride (20 c.c.) and heated at 75° under reflux for 5 hours; reducing action had then ceased. After neutralisation with barium carbonate the methyl-alcoholic solution, to which acetone (20 c.c.) had been added, was methylated twice with methyl sulphate (25 c.c.) and 30% sodium hydroxide solution (70 c.c.). The product was extracted with chloroform, the solvent removed, and the mobile syrup remethylated by two treatments with silver oxide (10 g.) and methyl iodide (30 c.c.). This resulted in the isolation of a syrup, which was distilled at 110° (bath temp.)/0·03 mm. to yield a mobile liquid (0·10 g.), $n_D^{20°}$ 1·4540; this was evidently a fully methylated fructoside.

Isolation of 1 : 3 : 4 : 5-Tetramethyl Fructose.—The tetramethyl methylfructoside was hydrolysed during 30 minutes with 3% hydrochloric acid, the solution neutralised with barium carbonate and evaporated to dryness under diminished pressure, and the residue extracted with ether. Removal of the solvent yielded a syrup, which partly crystallised in the square

plates of tetramethyl fructopyranose, m. p. 94—96° after recrystallisation from light petroleum, alone or in admixture with a specimen prepared directly from fructose, $[\alpha]_D^{20°}$ — 109° in water (*c*, 0·5).

Preparation of Trimethyl Glucosazone and Conversion into Trimethyl Fructose.—The tar (4 g.) obtained from the original methylation was found to contain OMe, 14·1%, and was subjected to two further methylations with methyl sulphate (20 c.c.) and 30% sodium hydroxide solution (50 c.c.) at 50° during 2 hours. Water (300 c.c.) was added, and the mixture cooled and filtered. The tarry residue was extracted with chloroform, the chloroform solution washed with water till neutral and dried with sodium sulphate, and the solvent removed under diminished pressure. The red syrup obtained (Found : OMe, 18·2%) was subjected to three methylations with methyl iodide (30 c.c.) and silver oxide (10 g.), which was added in 1 g. portions every 20 minutes. The product was isolated as a red syrup in the usual way (Found : C, 63·3; H, 6·9; OMe, 22·6; N, 14·1. $C_{21}H_{28}O_4N_4$ requires C, 63·0; H, 7·0; OMe, 23·2; N, 14·0%).

The conversion of this *trimethyl glucosazone* (12 g.) into trimethyl glucosone and the reduction to the trimethyl fructose were carried out precisely as described for the monomethyl derivative, with the exception that the sugar was extracted from the mixture with barium acetate by means of boiling chloroform. This yielded a reducing syrup (0·4 g.), $[\alpha]_D^{20°}$ — 43° in methyl alcohol (*c*, 0·4), — 39° in water (*c*, 0·3) (Found : OMe, 42·2. Calc. for $C_9H_{18}O_6$: OMe, 41·9%).

Attempted Fructofuranoside Formation.—This was carried out as described for the monomethyl derivative, and the results are in Table IV. Blank experiments showed that the trimethyl methylfructopyranoside was 20% hydrolysed and the reducing value of free sugar increased by 20% under the experimental conditions. The figures recorded are for the relative amounts of the glycoside corrected by these factors.

TABLE IV.

	0·015*N*-Thiosulphate, c.c.				
Time.	Before hydrolysis.	After hydrolysis.	Free sugar, %.	Furanoside, %.	Pyranoside, %.
0	5·3	6·4	100	—	—
1 hr.	4·1	5·5	78	9	13
4 hrs.	3·8	4·9	72	6	23
19 ,,	3·2	4·4	62	8	30
24 ,,	3·1	4·2	58	8	34
48 ,,	2·4	3·4	45	8	47

At the end of 48 hours the solution was still reducing to Fehling's reagent. The results show in a roughly quantitative manner the gradual formation, as the available sugar disappears, of a glycoside which is hydrolysed only with difficulty. The values calculated as furanoside are uniformly low and constant, as in Table III, and are ascribed to impurity.

Preparation of 3 : 4 : 5-*Trimethyl Methylfructopyranoside and Methylation of the Product.*—The sugar (0·3 g.) was dissolved in 3% methyl-alcoholic hydrogen chloride (20 c.c.) and heated at 75° under reflux for 5 hours; reducing action had then ceased. After neutralisation with barium carbonate the methyl-alcoholic solution, to which acetone (20 c.c.) had been added, was methylated once with methyl sulphate and sodium hydroxide and once with silver oxide and methyl iodide. This resulted in the isolation of a syrup, which distilled at 110° (bath temp.)/ 0·03 mm. to yield a mobile liquid (0·15 g.), $n_D^{20°}$ 1·4520; this was evidently a fully methylated fructoside.

Isolation of 1 : 3 : 4 : 5-*Tetramethyl Fructose.*—This tetramethyl methylfructoside was hydrolysed as in the previous case to yield a syrup, which partly crystallised in the square plates of tetramethyl fructopyranose, m. p. 94—96° alone or in admixture with a specimen prepared directly from fructose.

Thanks are expressed to the Earl of Moray Endowment and to Imperial Chemical Industries, Ltd., for grants.

KING'S BUILDINGS, UNIVERSITY OF EDINBURGH. [*Received, June 25th,* 1935.]

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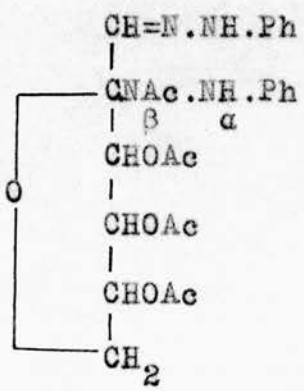
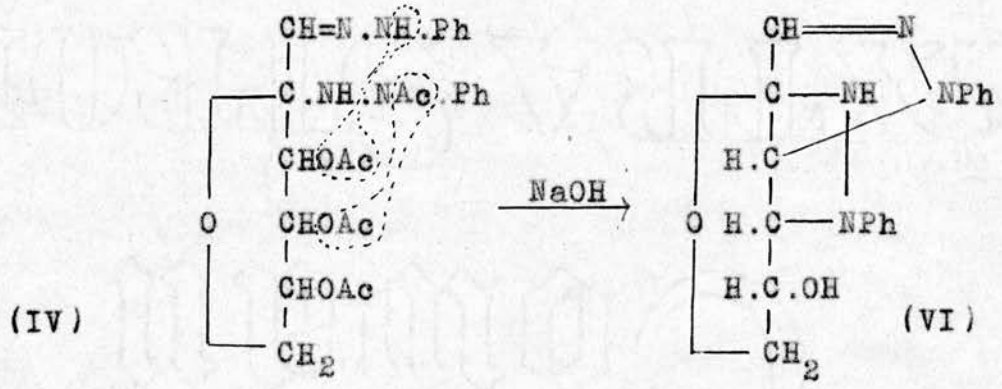
Further experiments designed to throw light on the reason for the discrepancy between the results of Wolfrom and those reported by Percival were described in 1937 by the present author (13). Owing to an error in the thermometer used for registering the temperature of the freezing mixture the Edinburgh experiments had been conducted at -15° to -18° instead of ca. -5°. It is apparent that the speed of deacetylation at the lower temperature will be much reduced and it was shown that for octa-acetyl lactose whereas 20 minutes was sufficient to eliminate all the acetyl residues at room temperature only 90% were removed at -20° in 2 hours. Since at -5° Wolfrom's results were confirmed, complete agreement was secured on the experimental facts. At the same time it was emphasised that it does not follow that the compounds in question do not contain N-acetylated phenylhydrazide groups. Wolfrom and his co-workers founded their method on the difference in the ease of deacetylation of acetanilide and methylacetanilide, and sugar acetates, the former being untouched during contact with N/10-sodium hydroxide at room temperature for 24 hours. The compounds α-acetylphenylhydrazine, β-acetylphenylhydrazine, αβ-diacetylphenylhydrazine and benzaldehyde-α-acetylphenylhydrazone were studied with regard to their/

their ease of deacetylation. These were shown to be hydrolysed much more readily with alkali than N-acetylated amines but to a varying extent; β-acetylphenylhydrazine loses 50% of its acetyl content during 2 hours at room temperature and diacetyl phenylhydrazine 38% in 5 minutes, ca 50% in 10 minutes although deacetylation is not complete until about 42 hours. At -17° 2 hours sees the removal of about 50% of the acetyl content. Since α-acetyl phenylhydrazine is not completely deacetylated during 47 hours at room temperature it seems reasonable to suppose that it is the β-acetyl group which is most readily eliminated and this receives support from the fact that benzaldehyde α-acetyl phenylhydrazone is also deacetylated with difficulty, being only 70% complete in 20 hours. It is not possible therefore to differentiate sharply between OAc and NH.NAc especially where the N-acetyl group is contained in the β-position in a hydrazide structure. It is tempting to conclude from this that the acetylated osazones carry the acetyl group on a nitrogen atom in the β-position as shown (V).



(V)

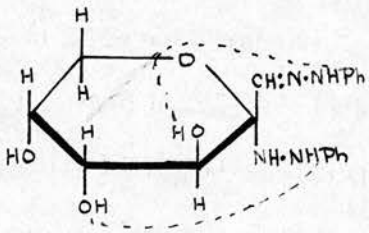
In 1936 E.C.V.Percival (5) by deacetylating glucosazone and galactosazone tetra-acetates at room temperature isolated a crystalline dianhydrohexosazone $C_{18}H_{18}O_2N_4$, products identical in every respect being isolated from both the above sources. The hexosazone anhydride contained but one hydroxyl group as shown by the isolation of a monoacetate and a monomethyl ether, formed an insoluble dibromide indicating the presence of a double bond, and was extremely stable since it could be crystallised from concentrated hydrochloric acid. The formation of this substance was accounted for as follows:



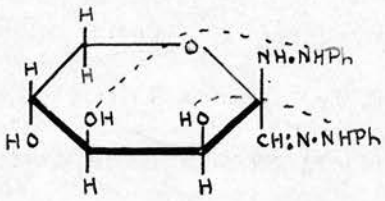
(IV) (VI)

Although of course it might equally well arise from (V). It was necessary to assume a Walden inversion at C_4 on deacetylation and ring formation in order to account for the isolation of the same anhydride from both glucose and galactose derivatives. From an inspection of models it appeared that only the α-fructopyranose derivative (VII) and the β-tagatopyranose (VIII)/

(VIII) can yield structures of the type (VI) with any ease.



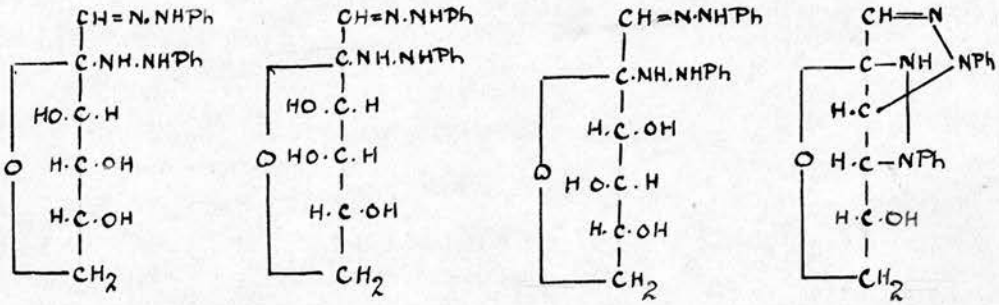
VII



VIII

Further support for the formulation of glucosazone as containing a fructopyranose ring was forthcoming by the observation of Diels, Cluss, Stephan and König in 1938 (14) that glucosazone would not react with triphenyl-chloromethane, a fact which was taken as evidence of the absence of a free primary alcohol group. Conclusive evidence was also obtained in the same year by E.G.V.Percival (15) that the dianhydrohexosazone described above likewise contained no primary alcohol residue for it yielded a crystalline mono-p-toluenesulphonate which underwent no reaction when treated for long periods with sodium iodide in acetone at 100°. This reaction was introduced by Oldham and Rutherford (16) as a test for primary alcohol residues and has been applied by many workers with success. It was also established beyond doubt that the anhydride formation is concerned with the hydroxyl/

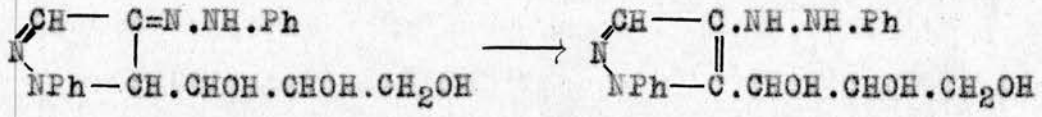
hydroxyl groups on C_3 and C_4 since d-gulosephenylosazone tetra-acetate yielded the same dianhydrohexosazone as previously isolated from the tetra-acetates of d-galactosazone and -glucosazone. From this result it follows that Walden inversion may also take place either on C_3 or C_4 or on both in order to arrive at the most stable arrangement of the three rings, although in which case this takes place is unknown, that is it cannot be decided whether the anhydride is a derivative of d-fructo-, d-tagato-, d-sorbo-, or d-psicopyranose. Tetra-acetyl l-sorbosazone on deacetylation yielded the l-enantiomorph of the dianhydrohexosazone as would be expected from the above observations.



d-Glucosazone d-Galactosazone d-Gulosazone d-Dianhydrohexosazone

Previous to the isolation of the dianhydrohexosazone Diels and Meyer in 1935 (17) reported the isolation from glucosazone and galactosazone, by treatment with alcoholic sulphuric acid, of a monoanhydro-hexosazone/

zone which they considered to be 3:6-anhydroglucosazone. This formulation was corrected in 1936 by Diels, Meyer and Onnen (18), in which it is postulated that the 4-pyrazoline derivative (IX) isomerises into the pyrazole structure (X).



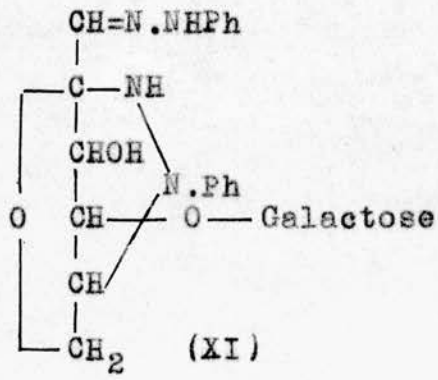
(IX) (X)

The German authors claim to have isolated triacetates from their anhydrides, but this would not invalidate the assumption of a cyclic structure for the monoanhydro-osazones since they point out that they made no attempt to discriminate between O-acetyl and N-acetyl groups; furthermore the conditions for acetylation they employed were drastic. Their isolation of dibenzoates suggested at once that a cyclic structure was present in derivatives of this type also. Accordingly the acetylation of these monoanhydro-osazones was undertaken by the present author in 1937 (13) and diacetates were obtained in both cases so that it is evident that these monoanhydro-osazones are also cyclic.

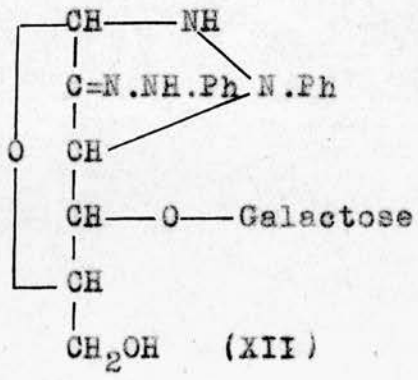
Diels and his co-workers had also reported the isolation by the action of sulphuric acid in alcohol of monoanhydro-lactosazone, -cellobiosazone -xylosazone, -arabinosazone/

-arabinosazone and dianhydro-maltosazone and it may be recalled that Emil Fischer (2) had reported on the isolation of anhydro-lactosazone by this method nearly fifty years earlier. The present author therefore attempted to extend the possibility of anhydride formation in the sugar osazones, by the deacetylation method, to the acetates of lactose, maltose, d-xylose, l-arabinose and l-rhamnose acetates. Positive results were obtained only in the first two cases.

Deacetylation of lactosazone hepta-acetate yielded the same anhydro-lactosazone as that described by Fischer and by Diels and Meyer; the compound proved to be a hydrated anhydride which on acetylation yielded a penta-acetyl monoanhydro-lactosazone which crystallised with one molecule of benzene. This being the case an oxide ring must be present in this monoanhydro-lactosazone which may be formulated as (XI) or (XII).



(XI)



(XII)

Maltosazone gave a crystalline hepta-acetate

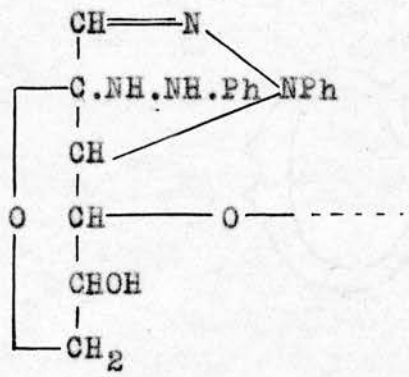
which/

which gave two distinct products on deacetylation:
(A) $C_{24}H_{30}O_8N_4$. long, lemon yellow needles, m.p. 245°, $[\alpha]_D^{20°}$ +58° in pyridine (c, 0.4), and
(B) $C_{24}H_{34}O_{10}N_4$. pointed yellow plates, m.p. 194°, $[\alpha]_D^{20°}$ +160° in pyridine (c, 0.2). These substances were not interconvertible by any method of dehydration or hydration attempted and the specific rotation indicates that they are essentially structurally different. Both yielded penta-acetates, $C_{34}H_{40}O_{13}N_4$, of markedly different properties and the conclusion was reached that these monoanhydro-maltosazones were isomers corresponding to the formulae proposed for monoanhydro-lactosazone. In any event the oxide-ring structure persists.

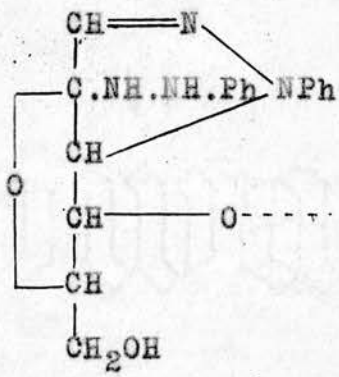
In conclusion mention may be made of an extension of this work by J.R.Muir and E.G.V.Percival in 1940 (19).

Cellobiosazone was shown to yield a crystalline hepta-acetate which yielded a monoanhydro-cellobiosazone hydrate, m.p. 218°, which gave a penta-acetate on acetylation and from which the anhydride was recoverable by deacetylation; it was shown that the method of Diels undoubtedly gave the same product although the melting point recorded by Diels, Meyer and Onnen (225-245°) (18) could not be reached. Cellobiosazone/

biosazone anhydride must therefore possess an oxide-ring structure, and one of the types of structure proposed for lactosazone or maltosazone anhydride would suit. The possibilities of 1:3-anhydro (2:6-oxide) (XIII) and 1:3-anhydro (2:5-oxide)(XIV) cannot be excluded however.



(XIII)



(XIV)

Muir and Percival failed to isolate anhydro-compounds by deacetylating gentiobiosazone and melibiosazone hepta-acetates, a result similar to that obtained by the present author (13) for the acetates of arabinosazone, xylosazone and rhamnosazone. When C_6 is substituted or absent no ketopyranose structure is possible and if this aids the crystallisation of the anhydrides this would explain the facts. It was pointed out that there is double the number of theoretical possibilities for anhydride formation when the hydroxyl group on C_6 is substituted (as in gentiobiose) rather than that on C_4 (in maltose), namely three pyranose and five furanose monoanhydrides, so it is likely that/

that mixtures result in these cases.

In the same paper it is shown that galactosazone fails to react with triphenylchloromethane which is further evidence for the absence of a primary alcohol group and methylation of galactosazone yielded a crystalline trimethyl galactose methylphenyl-phenylosazone which further supports the cyclic structure for galactosazone which was inferred by its behaviour on acetylation and deacetylation.

From the foregoing it is seen that in the past seven years our knowledge of the constitution of osazones has been added to to a considerable extent and there would seem to be no reasonable doubt as to the existence of a 2:6-oxide ring in the hexosazones. The fact that these compounds form anhydrides so readily even when Walden inversion must necessarily accompany the process in some cases was unforeseen and the work described in the next section was initiated in an attempt to discover more about this reaction.

SUGAR OSAZONES AND THEIR ANHYDRIDES

BY
E. E. PERCIVAL
AND
E. G. V. PERCIVAL

Reprinted from the Journal of the Chemical Society,
July, 1937.

Reprinted from the Journal of the Chemical Society, 1937.

268. *Sugar Osazones and their Anhydrides.*

By E. E. Percival and E. G. V. Percival.

This paper deals with further investigations on the production of anhydro-osazones by the deacetylation of osazone acetates. A monoanhydro-lactosazone and two isomeric monoanhydro-maltosazones are described, all of which yield penta-acetates and therefore possess a pyranose ring structure in addition to the pyrazolidine or pyridazine ring. The monoanhydro-glucosazone and -galactosazone described by Diels and his co-workers also appear to contain an oxide ring, since they yield diacetates on acetylation.

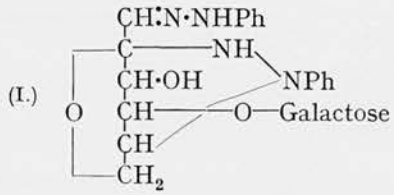
It is pointed out as a result of further work on the differential deacetylation of osazone acetates that this titration method cannot be used as evidence of the cyclic structure or otherwise of such derivatives.

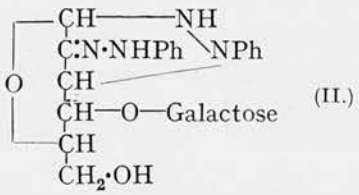
It was shown by one of us (J., 1936, 1770) that the deacetylation of the acetyl derivatives of glucosephenylosazone and galactosephenylosazone gave rise to the same dianhydrohexosazone, for which a structure was proposed incorporating a pyrazoline, a pyrazolidine, and a pyranose ring. Previously Diels and Meyer (*Annalen*, 1935, **519**, 157) and later Diels, Meyer, and Onnen (*ibid.*, 1936, **525**, 94) had reported the isolation of monoanhydro-glucosazone, -galactosazone, -lactosazone, -xylosazone, -arabinosazone, -cellobiosazone, dianhydro-maltosazone and other similar derivatives by the action of sulphuric acid in alcoholic solution on the osazones. Emil Fischer in his classical paper on osazone formation (*Ber.*, 1887, **20**, 830) had also reported the isolation of anhydrolactosazone by this method.

We have now investigated further the possibility of anhydride formation in the sugar osazone series by the deacetylation method for the acetates of the phenylosazones of lactose, maltose, *d*-xylose, *l*-arabinose, and *l*-rhamnose, as well as for certain phenylhydrazones and methylphenylhydrazones, but positive results have been obtained only in the case of the first two substances.

Lactosazone yields an amorphous *hepta-acetate*, which on deacetylation reverts to the same anhydro-derivative (m. p. 232°) as that described by Fischer and by Diels and Meyer (*locc. cit.*). Ultimate analysis reveals the fact that the formula must be represented as $C_{24}H_{32}O_9N_4$, and since it is not identical with lactosazone it appears to be a hydrated anhydride. The corresponding acetyl derivative crystallises with one molecule of benzene and appears to be a *penta-acetyl monoanhydrolactosazone*, the analytical figures not being in agreement with those required by a dianhydrolactosazone tetra- or penta-acetate, or by monoanhydrolactosazone hexa-acetate. It is probable, therefore, that a pyranose ring

structure is present in this monoanhydrolactosazone and it can accordingly be formulated as either (I) or (II).





It should be noted that the above formulæ are not stereochemical.

Crystalline *maltosazone hepta-acetate* gave two distinct products on deacetylation: (A) $C_{24}H_{30}O_8N_4$, long, lemon-yellow needles, m. p. 245°, $[\alpha]_D^{20°}$ + 57·6° in pyridine (*c*, 0·38), and (B) $C_{24}H_{34}O_{10}N_4$, pointed, yellow plates, m. p. 194°, $[\alpha]_D^{20°}$ + 160° in pyridine (*c*, 0·23). Since by no method of dehydration or hydration attempted was it possible to interconvert (A) and (B), coupled with the evidence of the specific rotations, it is necessary to conclude that the two derivatives are structurally different. The possibility too that (B) is maltosazone hydrate can be ruled out on the basis of the rotational evidence and the fact that on acetylation (B) yields a *penta-acetate*, $C_{34}H_{40}O_{13}N_4$, and not maltosazone hepta-acetate. Acetylation of (A) also yields an amorphous *penta-acetate* of the same composition but with properties markedly different from those of acetylated (B). The simplest explanation available is that (A) and (B) are the isomeric ring forms corresponding to formulæ (I) and (II) suggested for the anhydrolactosazone, but it has not yet been found possible to distinguish between the two forms. It is interesting to note that, although by the deacetylation method glucosazone yields a dianhydrohexosazone and maltosazone and lactosazone monoanhydrides, yet Diels and Meyer (*loc. cit.*) by their method obtained a monoanhydroglucosazone and a dianhydromaltosazone.

In a paper (J., 1935, 1398) a structure for glucosazone was proposed embodying a fructopyranose ring on the basis of methylation experiments. There seems to be no reason why this should not hold for the anhydro-osazones of the disaccharides now considered, but since no experimental proof is yet available for these derivatives it is necessary to suspend judgment on the location of the oxide ring which is undoubtedly present.

The *triacetates* of *d*-xylosazone, *l*-arabinosazone and *l*-rhamnosazone were prepared, but despite repeated attempts crystalline anhydro-osazones could not be obtained by the deacetylation method, although Diels, Meyer, and Onnen (*loc. cit.*) found it possible with acidified alcohol to prepare monoanhydrides of xylosazone and arabinosazone.

Since all the osazone anhydrides prepared by deacetylation appeared to possess an oxide ring structure, it seemed of interest to investigate whether the monoanhydrides of Diels were of the same type. Accordingly his monoanhydro-glucosazone and -galactosazone were prepared and acetylated. The former yielded an amorphous *diacetate*, $[\alpha]_D^{17°}$ — 125°, and a crystalline *diacetyl monoanhydrogalactosazone*, $[\alpha]_D^{17°}$ + 64°, was also isolated. These results would suggest that these monoanhydrides possess an oxide ring structure. This is supported by the observation of Diels, Meyer, and Onnen (*loc. cit.*) that they yield dibenzoates, but these authors also report the isolation of triacetates. The latter observation may be due to acetylation on one of the nitrogen atoms of the hydrazone residue due to the employment of vigorous methods of acetylation. No specific rotations were recorded for these derivatives.

Fructosemethylphenylosazone tetra-acetate on deacetylation yielded the original osazone as would be expected, since no α-hydrogen atom is available for anhydride formation; Diels and Meyer (*loc. cit.*) record a similar observation for their acid alcohol method. Incidental to the preparation of fructosemethylphenylosazone a compound which appears to be a *fructosemethylphenylhydrazone*, m. p. 170°, $[\alpha]_D^{17°}$ — 253°, yielding a *penta-acetate*, m. p. 121°, $[\alpha]_D^{17°}$ + 86·5°, was isolated. Ofner (*Monatsh.*, 1905, **26**, 1165) has described a fructosemethylphenylhydrazone of m. p. 116—120°. It was not found possible to isolate an anhydro-compound from the acetate of glucosephenylhydrazone or from *glucosemethylphenylhydrazone penta-acetate*.

Because of the importance of being able to distinguish between *O*-acetyl and *N*-acetyl groups in this work and because the results in a previous paper (Percival, *loc. cit.*) did not agree with those of Wolfrom, Konigsberg, and Soltzberg (*J. Amer. Chem. Soc.*, 1936, **58**, 490) an extensive survey of the method previously described has been carried out. Repetition of the work on tetra-acetyl galactosazone and tetra-acetyl glucosazone with the conditions of temperature prescribed by Kunz and Hudson (*J. Amer. Chem. Soc.*, 1926, **48**, 1982) indicated the presence of *ca.* 30—31% of *O*-acetyl group, and we are now in complete agreement with Wolfrom and his co-workers on the experimental facts. The reason for the discrepancy was the inaccuracy below 0° of the thermometer used for registering the temperature of the freezing mixture, with the result that the earlier experiments had been conducted at — 15° to — 18° instead of *ca.* — 5°. Whereas at the lower temperature results were obtained, and these are now confirmed, corresponding to the removal of but three acetyl residues, it is apparent that the speed of deacetylation under these conditions will be much reduced, and experiments with octa-acetyl lactose reveal that, although 20 minutes at room temperature is sufficient to eliminate all the acetyl residues, at — 20° only about 90% is removed in 2 hours, so that the agreement may be fortuitous. It must be emphasised, however, that it does not follow that the compounds in question do not contain *N*-acetyl groups, and are therefore acyclic. It is clear that it is not sufficient to compare *O*-acetylated compounds with such derivatives as acetanilide and methylacetanilide, which are untouched during 24 hours with *N*/10-sodium hydroxide at room temperature, since the compounds under review, if cyclic, will be acetylated hydrazides. Accordingly the ease of deacetylation of α-acetylphenylhydrazine, β-acetylphenylhydrazine, αβ-diacetylphenylhydrazine and benzaldehyde-α-acetylphenylhydrazone was studied. The general conclusion emerges that such compounds are hydrolysed much more easily than *N*-acetylated amines, but to a varying extent; *e.g.*, β-acetylphenylhydrazine requires but 2 hours at room temperature under the prescribed conditions to lose 50% of its acetyl residues and diacetylphenylhydrazine loses almost the same proportion in 10 minutes, although benzaldehyde-α-acetylphenylhydrazone only loses 13% and α-acetylphenylhydrazine 7% in 2 hours. It is therefore clear that a sharp differentiation between NH·NAc and OAc is difficult by the method proposed and that the question of the structure of the osazone acetates cannot yet be regarded as settled.

Experimental.

Acetylation of Lactosephenylosazone.—The method described for the acetylation of dianhydrohexosazone (J., 1936, 1773) was employed, giving, in almost quantitative yield, an amorphous yellow powder, which was washed, dried, dissolved in benzene, and precipitated with light petroleum (b. p. 40—60°) to yield a pale yellow solid, m. p. 105—110°, $[\alpha]_D^{20°}$ + 27° in chloroform (*c*, 0·28) (Found: C, 56·6; H, 5·6; CH_3·CO, 35·7; N, 7·1. $C_{38}H_{46}O_{16}N_4$ requires C, 56·0; H, 5·65; CH_3·CO, 37·0; N, 6·9%).

Conversion into Anhydrolactosephenylosazone.—Hepta-acetyl lactosephenylosazone (4 g.), dissolved in acetone (180 c.c.) and water (100 c.c.), was mixed with sodium hydroxide solution (44 c.c., 8%) at room temperature and kept for 21 hours. The resulting solution was neutralised with sulphuric acid and diluted with acetone until the precipitation of sodium sulphate was complete. This was removed by filtration, and the acetone by distillation. Yellow needles were deposited, which were filtered off from the hot solution; a further quantity of needles was deposited from the filtrate on standing. Recrystallisation from hot pyridine–alcohol, followed by the addition of water, gave light yellow, fan-like needles (1 g.), m. p. 231—232° (not depressed by Diels's anhydrolactosazone, m. p. 230°), $[\alpha]_D^{20°}$ — 147° in methyl alcohol (*c*, 0·18) (cf. Diels's anhydrolactosazone, $[\alpha]_D^{20°}$ — 146° in methyl alcohol; *c*, 0·19) (Found: C, 55·2; H, 6·1; N, 11·6. Calc. for $C_{24}H_{32}O_9N_4$: C, 55·4; H, 6·2; N, 10·8%).

Preparation of Anhydrolactosephenylosazone Penta-acetate.—Anhydrolactosephenylosazone (0·4 g.) was acetylated as described for the acetylation of lactosephenylosazone. On pouring into water a yellow precipitate was obtained. Rosettes of shining yellow needles were obtained by solution in warm benzene, followed by the addition of light petroleum (b. p. 40—60°) until turbidity was almost reached (yield 0·5 g.); m. p. 115—117°, $[\alpha]_D^{20°}$ — 102° in acetone (*c*, 0·4) [Found: C, 60·3; H, 5·82; CH_3·CO, 29·0 (titrn.), 28·0 (Freudenberg); N, 7·1. $C_{40}H_{46}O_{13}N_4$ requires C, 60·7; H, 5·9; CH_3·CO, 27·3; N, 7·1%]. Deacetylation of the *penta-acetate* gave the original anhydrolactosazone, m. p. 232°, $[\alpha]_D^{17°}$ — 147° in methyl alcohol (*c*, 0·2).

Acetylation of Maltosephenylosazone.—Maltosephenylosazone was acetylated as described above for lactosephenylosazone. The *product*, obtained in quantitative yield, was recrystallised from alcohol and water to yield rosettes of yellow needles, m. p. 162°, $[\alpha]_D^{20°}$ + 41° in chloroform (*c*, 0·515) (Found: C, 55·7; H, 5·6; CH_3·CO, 37·0; N, 6·8. $C_{38}H_{46}O_6N_4$ requires C, 56·0; H, 5·65; CH_3·CO, 37·0; N, 6·9%).

Preparation of Anhydromaltosephenylosazone.—Hepta-acetyl maltosephenylosazone (9·5 g.) was deacetylated as described above for hepta-acetyl lactosephenylosazone. A yellow precipitate (A) was again deposited in the hot solution and a further quantity of precipitate (B) in the filtrate on standing. The precipitate (A) was recrystallised in the same way as the lactose compound to give long, pale yellow needles (0·3 g.), m. p. 245—246°, mixed m. p. with anhydroglucosephenylosazone (m. p. 230—232°) 224—226°; $[\alpha]_D^{20°}$ + 58° in pyridine (*c*, 0·382) (Found: C, 57·6; H, 6·0; N, 10·9. $C_{24}H_{30}O_8N_4$ requires C, 57·4; H, 6·0; N, 11·2%).

The precipitate (B) was similarly treated to yield bright yellow, pointed plates mixed with a small quantity of the light yellow needles (0·05 g.). It was found that the needles were deposited while the recrystallisation solution was warm and separation was effected by filtration of the hot solution. Any plates adhering to the needles could be removed by washing with alcohol. The addition of a further quantity of water was sometimes necessary to ensure complete deposition of the plates (2·3 g.), m. p. 194°; mixed m. p. with maltosephenylosazone (m. p. 196—199°) 165°; $[\alpha]_D^{20°}$ + 160° in pyridine (*c*, 0·23), + 90° in methyl alcohol (*c*, 0·552), + 92° in 6 : 4 alcohol–pyridine (*c*, 0·3) (Found: C, 53·6; H, 6·3; N, 10·7. $C_{24}H_{34}O_{10}N_4$ requires C, 53·5; H, 6·3; N, 10·4%).

Acetylation of Anhydromaltosephenylosazone.—(a) *Needle form.* The needles (0·27 g.) were acetylated as described above, slight warming being necessary to obtain solution of the crystals. On pouring into water an orange-red precipitate was obtained, which defied all attempts at crystallisation. The best method of purification was solution in benzene, followed by precipitation with light petroleum (b. p. 60—80°) to give a pale fawn, amorphous *penta-acetate* (0·4 g.), $[\alpha]_D^{20°}$ + 90·7° in acetone (*c*, 0·275) [Found: C, 56·7; H, 5·6; CH_3·CO (Freudenberg), 30·0; N, 7·3. $C_{34}H_{40}O_{13}N_4$ requires C, 57·3; H, 5·6; CH_3·CO, 30·2; N, 7·9%]. All attempts to obtain a crystalline anhydro-compound or to regenerate the original material by deacetylation of the above acetate failed.

(b) *Plate form.* The plates (1·3 g.) were acetylated as described above to yield a pale yellow solid. Recrystallisation from alcohol gave an amorphous *penta-acetate* (1·8 g.), m. p. 110—112°, $[\alpha]_D^{20°}$ + 150° in acetone (*c*, 0·29) [Found: C, 56·8; H, 5·5; CH_3·CO, 30·5 (titrn.), 30·8 (Freudenberg); N, 7·5. $C_{34}H_{40}O_{13}N_4$ requires C, 57·3; H, 5·6; CH_3·CO, 30·2; N, 7·9%]. Deacetylation of the above acetate (1 g.) and treatment in the usual manner gave the original pure plates (0·25 g.), m. p. 194°, $[\alpha]_D^{18°}$ + 160° in pyridine (*c*, 0·243).

d-*Xylosazone Triacetate*, l-*Arabinosazone Triacetate, and* l-*Rhamnosazone Triacetate.*—The pure osazone (1 g.) was dissolved in pyridine (5·5 c.c.) and acetic anhydride (2 c.c.) and kept for 36 hours. The solid obtained on pouring into water was recrystallised from aqueous ethyl alcohol.

d-*Xylosazone triacetate* crystallised in clumps of needles, m. p. 116—117°, $[\alpha]_D^{16°}$ − 46° in chloroform (*c*, 0·3) [Found: C, 60·9; H, 5·9; CH_3·CO, 29·9 (Freudenberg), 27·9 (titrn.); N, 12·7. $C_{23}H_{26}O_6N_4$ requires C, 60·7; H, 5·8; CH_3·CO, 28·4; N, 12·3%].

l-*Arabinosazone triacetate* was similar in appearance to the corresponding xylose derivative; it had m. p. 114°, $[\alpha]_D^{16°}$ *ca.* + 5° in chloroform (*c*, 0·3) [Found: C, 61·2; H, 6·1; CH_3·CO, 30·0 (Freudenberg), 28·0 (titrn.); N, 12·5. $C_{23}H_{26}O_6N_4$ requires C, 60·7; H, 5·8; CH_3·CO, 28·4; N, 12·3%].

l-*Rhamnosazone triacetate* was obtained as an amorphous yellow solid, m. p. 75°, $[\alpha]_D^{18°}$ + 52° in chloroform (*c*, 0·4) [Found: C, 61·4; H, 6·0; CH_3·CO (titrn.), 28·4; N, 12·2. $C_{24}H_{28}O_6N_4$ requires C, 61·5; H, 6·0; CH_3·CO, 27·6; N, 12·0%].

Deacetylation of these compounds according to the conditions previously described gave ill-defined brownish-yellow solids which could not be obtained crystalline.

Monoanhydroglucosazone and Monoanhydrogalactosazone Diacetates.—Diels and Meyer's method (*loc. cit.*) was used to prepare monoanhydroglucosazone, m. p. 177°, $[\alpha]_D^{17°}$ − 154° in methyl alcohol (*c*, 0·45) (Found: C, 63·2; H, 6·1; N, 16·3. Calc. for $C_{18}H_{20}O_3N_4$: C, 63·5; H, 5·9; N, 16·5%), and monoanhydrogalactosazone, m. p. 217°, $[\alpha]_D^{20°}$ + 28° in methyl alcohol (*c*, 0·3) (Found: C, 63·1; H, 6·0; N, 16·2. Calc. for $C_{18}H_{20}O_3N_4$: C, 63·5; H, 5·9; N, 16·5%). Both these derivatives (1 g.) were acetylated with pyridine (8 c.c.) and acetic anhydride (3·5 c.c.) during 3 days at room temperature and the acetates were isolated by pouring into water. *Monoanhydroglucosazone diacetate* was obtained as a yellow amorphous powder, m. p. 70°,

$[\alpha]_D^{17°}$ — 125° in chloroform (*c*, 0·3) [Found: C, 62·0; H, 5·8; CH_3·CO, 21·2 (Freudenberg), 21·4 (titrn.); N, 12·8. $C_{22}H_{24}O_5N_4$ requires C, 62·3; H, 5·7; CH_3·CO, 20·3; N, 13·2%]. *Monoanhydrogalactosazone diacetate* crystallised in yellow needles, m. p. 86°, $[\alpha]_D^{18°}$ + 64° in chloroform (*c*, 0·2) [Found: C, 61·9; H, 5·8; CH_3·CO, 21·6 (Freudenberg), 21·0 (titrn.); N, 13·5. $C_{22}H_{24}O_5N_4$ requires C, 62·3; H, 5·7; CH_3·CO, 20·3; N, 13·2%].

Fructosemethylphenylosazone Tetra-acetate.—The instructions of Ofner (*Ber.*, 1904, **37**, 3362) were followed, but the product was invariably the *methylphenylhydrazone* described below. Neuberg's method (*Ber.*, 1902, **35**, 959), however, gave the methylphenylosazone, m. p. 156°, $[\alpha]_D^{17°}$ + 90° in pyridine–alcohol (4 : 6) (*c*, 0·4). Acetylation according to the usual method gave a yellow crystalline acetate in quantitative yield, m. p. 128°, $[\alpha]_D^{17°}$ — 435° in chloroform (*c*, 0·4), — 236° in 95% alcohol (*c*, 0·2) (cf. Engel, *J. Amer. Chem. Soc.*, 1935, **57**, 2419) (Found: C, 60·9; H, 6·2; CH_3·CO, 30·9; N, 10·0. Calc. for $C_{28}H_{34}O_8N_4$: C, 60·6; H, 6·2; CH_3·CO, 31·0; N, 10·1%).

Fructosemethylphenylhydrazone.—Ofner's method (*loc. cit.*) for the preparation of the osazone readily gave a colourless crystalline derivative, which on recrystallisation yielded prisms, m. p. 170°, $[\alpha]_D^{17°}$ — 253° in pyridine–alcohol (4 : 6) (*c*, 0·6) (Found: N, 10·3. $C_{13}H_{20}O_5N_2$ requires N, 9·9%).

Fructosemethylphenylhydrazone Penta-acetate.—The methylphenylhydrazone (1 g.) was kept with acetic anhydride (3 c.c.) and pyridine (6 c.c.); after 2 days the mixture was poured into water, and the solid recrystallised from 50% aqueous alcohol to yield colourless plates of the *penta-acetate*, m. p. 121°, $[\alpha]_D^{17°}$ + 86·5° in chloroform (*c*, 0·9) (Found: C, 56·0; H, 6·1; CH_3·CO, 42·8; N, 6·4. $C_{23}H_{30}O_{10}N_2$ requires C, 55·85; H, 6·1; CH_3·CO, 43·5; N, 5·7%).

Substance.	Temp.	Time (hrs.).	% CH_3·CO.
Galactosazone tetra-acetate (32·5)	−22°	0·5	22·2
	−22	1·33	21·2
	−20 → −14	2·0	23·0
	−20 → −18	2·33	25·0*
	−20 → −10	3·17	26·6
	−23 → −21	3·5	28·3
	−20 → −17	4·0	27·4
	−20 → −18	6·42	29·0
	− 4 → − 6	2·0	29·9*
	− 4 → − 6	3·0	31·9
	+ 17	2·0	33·4*
	+ 17	2·67	34·0
	+ 17	3·0	33·6
	+ 17	4·0	33·5*
Lactose octa-acetate (50·0)	−19 → −18	0·2	24·4
	−19 → −18	0·45	30·0
	−19 → −18	0·83	42·9
	−19 → −18	1·5	43·0
	−19 → −18	2·0	45·9
	−20 → −17	4·67	48·9
	+ 16	0·02	28·0
	+ 16	0·08	39·3
	+ 16	0·25	47·3
	+ 16	4·0	49·2*
Acetanilide (31·9)	+ 18	24·0	0
Methylacetanilide (28·9)	+ 18	24·0	0
Fructosemethylphenylosazone tetra-acetate (31·0)	−19	2·0	27·0
	−10	2·0	30·8
Fructosemethylphenylhydrazone penta-acetate (43·5)	−19	2·0	38·0
	− 5 → + 2	2·5	42·8
Xylosazone tri-acetate (28·4)	−12	2·0	18·0
	+17	2·0	26·0
Arabinosazone tri-acetate (28·4)	−13 → −10	2·0	20·0
	+17	2·0	28·0
"Dianhydro-hexosazone" mono-acetate (11·8)	−20°	2·0	7·0
	−10	2·0	9·0
	+17	7·0	11·9
Lactosazone hepta-acetate (37·0)	−20	2·0	24·1
	+17	2·0	36·0
Maltosazone hepta-acetate (37·0)	−10	2·0	32·0
	+16	2·0	37·0
	+16	4·0	37·3
Glucosephenylhydrazone penta-acetate (44·8)	−10 → −15	2·0	32·0
	+16	3·0	43·4
Glucosemethylphenylhydrazone penta-acetate (43·6)	−17 → −14	0·5	22·3
	−17 → −14	1·0	34·6
	−17 → −14	2·0	37·9
	−17 → −10	4·0	39·8
	−17 → − 8	6·5	42·3
	− 0 → − 5	14·0	40·5
	+16	3·0	43·1
β-Acetylphenylhydrazine (28·7)	−20	2·0	8·0
	−4 → 0	2·0	9·0
	+17	2·0	14·0
	+17	5·0	22·7
	+17	17·0	24·5
α-Acetylphenylhydrazine (28·7)	+16	2·0	1·7
	+16	18·0	8·0
	+16	47·0	18·0
αβ-Diacetylphenylhydrazine (44·7)	−17 → −14	2·0	19·9
	+17	0·08	17·0
	+17	0·16	20·5
	+17	0·5	20·8
	+17	3·5	21·0
	+17	4·0	24·2
	+17	21·0	33·7
	+17	42·0	41·1
Benzaldehyde-α-acetylphenylhydrazone (18·1)	+17	2·0	2·3
	+17	20·0	13·0

Glucosephenylhydrazone Penta-acetate.—This derivative was prepared from glucosephenylhydrazone, m. p. 159°, $[\alpha]_D$ — 82° in water (*c*, 0·5), by the method of Behrend and Reinsberg

(*Annalen*, 1910, **377**, 189). The needles had m. p. 152°, $[\alpha]_D^{17°}$ − 10·4° in pyridine (*c*, 0·5) (Found: CH_3·CO, 43·4; N, 6·0. Calc. for $C_{22}H_{28}O_{10}N_2$: CH_3·CO, 44·8; N, 5·8%).

Glucosemethylphenylhydrazone Penta-acetate.—Glucosemethylphenylhydrazone (m. p. 131°; 3 g.) prepared according to the method of Ofner (*loc. cit.*) was acetylated by the addition of acetic anhydride (6 c.c.) and pyridine (16 c.c.) during 45 minutes with constant stirring; after 12 hours the mixture was poured into cold water. The white gummy solid obtained gave, on recrystallisation from alcohol, white shining prisms of the *penta-acetate* (2·5 g.), m. p. 113—114°, $[\alpha]_D$ + 157° in chloroform (*c*, 0·5) (Found: CH_3·CO, 43·1; N, 5·9. $C_{23}H_{30}O_{10}N_2$ requires CH_3·CO, 43·6; N, 5·7%).

Acetyl Estimations by Direct Titration.—For these experiments 0·10—0·15 g. of material was dissolved in acetone (35 c.c.), *N*/10-sodium hydroxide (25 c.c.) added, drop by drop in the case of the experiments in the cold, and the solution, after dilution, back-titrated with *N*/10-sulphuric acid and phenol-red. Controls were carried out on the acetone used in each experiment. Experiments using the quinhydrone electrode gave almost identical results in the cases marked *. The theoretical percentage of acetyl in each compound is given in parentheses after the name in the table.

Thanks are expressed to the Carnegie Trust for the award of a Teaching Fellowship (E. G. V. P.) and to the Earl of Moray Endowment for grants.

King's Buildings, University of Edinburgh. [*Received, June 2nd*, 1937.]

Printed in Great Britain by Richard Clay & Sons, Limited,
Bungay, Suffolk.

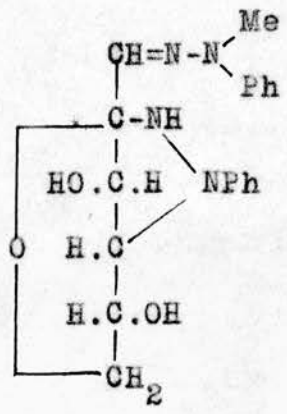
I_N_T_R_O_D_U_C_T_I_O_N

It has been pointed out previously that the stereochemical structure of the dianhydrohexosazone was left undecided although the isolation of the same product from d-gulosazone -glucosazone and -galactosazone made it clear that in appropriate circumstances inversion could take place at both C_3 and C_4, and probably also at C_2. By replacing each of the phenylhydrazone residues in turn, in the last two osazones, by phenylmethylhydrazine which has no hydrogen available for anhydride formation it was hoped to secure information from rotational data as to where inversion occurred. Another point of interest it was hoped to settle was the question whether the 2:6-oxide ring would be retained when C_2 carried a phenylmethylhydrazine residue, since Wolfrom and Christman (20) demonstrated in 1931 that galactosephenylmethylhydrazone is acyclic.

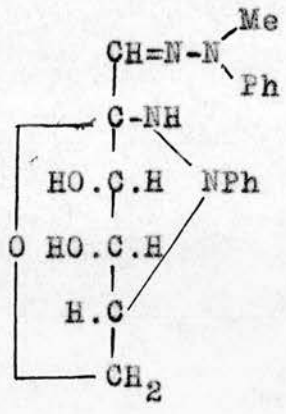
Finally this author had described in 1937 (13) a fructosephenylmethylhydrazone, m.p. 170°, $[\alpha]_D^{20°}$ -253° markedly different from that first described by Ofner (21). These substances were reinvestigated by W.J.Heddle and E.G.V.Percival (22) who suggested that the former product was cyclic and that of Ofner acyclic, and it was hoped to throw more light on this

Discussion of Results

Galactose-phenylmethylhydrazone on treatment with phenylhydrazine readily yielded a new osazone, galactosephenylmethyl-phenylosazone, m.p. 178°, $[\alpha]_D^{17°}$ +98°. Acetylation yielded a crystalline tetra-acetate, m.p. 183°, $[\alpha]_D^{13°}$+85° which was converted by deacetylation into a monoanhydrogalactose-phenylmethyl-phenylosazone, m.p. 172°, $[\alpha]_D^{13°}$+100°. This compound contained two free hydroxyl groups for it yielded a crystalline di-acetate and a di-p-toluenesulphonate. This compound did not react with sodium iodide in acetone at 100° and it was concluded therefrom that a primary alcohol residue was absent. The structure (XV) may be assigned to this anhydride; the alternative structure (XVI) can be ruled out be-



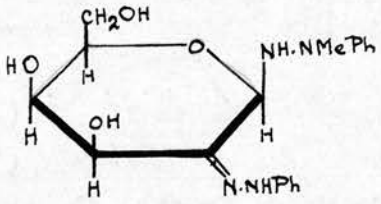
(XV)



(XVI)

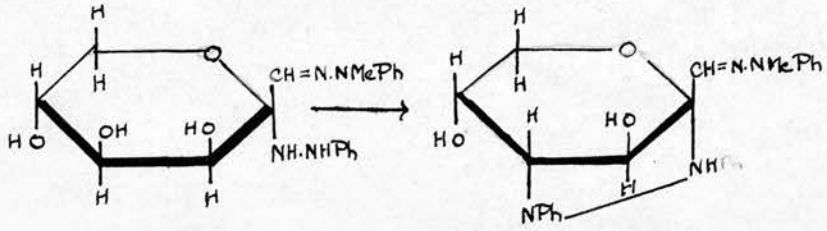
cause the anhydride failed to condense with acetone. It/

It is interesting to note that if the galactosephenylmethyl-phenylosazone possessed structure (XVII) containing a 1:5-oxide ring great strain would be imposed on anhydride formation owing to the rigidity conferred by the presence of the double bond on C_2.



(XVII)

From the similarity in the specific rotations of galactosephenylmethyl-phenylosazone and its anhydro-derivative it would seem likely that the configuration on C_2 is unchanged on anhydride formation, although if this is a (XVIII) inversion is necessary on C_4 and the anhydride (XIX) is related to fructopyranose.



(XVIII) (XIX)

Unfortunately this chapter could not be completed by the preparation of the isomeric galactosephenyl-phenylmethylosazone/

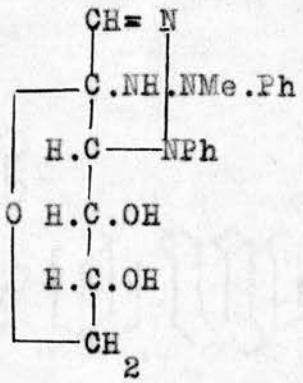
phenylmethylosazone. Treatment of galactosephenylhydrazone with phenylmethylhydrazine invariably produced the highly insoluble galactosephenylmethylhydrazone (70%), together with the same osazone (12%) as that described above, which yielded the same anhydride.

It was found impossible in the glucose series also to prepare one of the desired mixed osazones. Votŏcek and Vondráček (23) claimed the isolation of glucosephenylmethyl-phenylosazone (B), m.p. 205°, from glucosephenylmethylhydrazone and phenylhydrazine, and of glucosephenyl-phenylmethylosazone (A), m.p. 192°; together with (B) from glucosephenylhydrazone (and fructosephenylhydrazone) and phenylmethylhydrazine. The experimental facts were verified but Votoček's structure for (B) is incorrect. Glucosephenylhydrazone and phenylmethylhydrazine gave a product (A), m.p. 194°, $[\alpha]_D^{17°}$ -53°⟶-6°, and (B), m.p. 202°, $[\alpha]_D^{17°}$ -60°⟶-15°. Glucosephenylmethylhydrazone and phenylhydrazine gave an osazone identical with (B) and Votoček's observation that more vigorous treatment gave glucosazone was confirmed.

When fructosephenylmethylhydrazone prepared according to Ofner (21) was treated with phenylhydrazine a mixed osazone identical with (B) was obtained whereas the fructosephenylmethylhydrazone, m.p. 170° (gave/

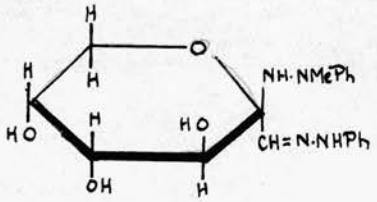
gave rise exclusively to (A). From this result it would appear that both (A) and (B) carry the phenylmethylhydrazine residue on C_2, although the formation of (B) from glucosephenylmethylhydrazone appears to indicate that it is on C_1 in this case. In one case therefore, a phenylmethylhydrazine residue has been displaced and transferred to another carbon atom. That displacement can occur is evident since glucosazone can be isolated when glucosephenylmethylhydrazone is heated with phenylhydrazine and a similar phenomenon was noted in the galactose series. The evidence suggests, however, that the exchange takes place in the case of glucosephenylmethylhydrazone since the osazone took seven minutes to appear when glucosephenylmethylhydrazone was employed, whereas when either of the fructosephenylmethylhydrazones were used only one minute was required under identical conditions. The conclusion reached, therefore, is that both (A) and (B) are glucose phenyl-phenylmethylosazones. That both have essentially the same structure is shown by the fact that they gave amorphous acetates of similar properties, $[\alpha]_D^{15°}$ -43°, which on deacetylation yielded the same monoanhydroglucosephenyl-phenylmethylosazone, m.p. 176-178°, $[\alpha]_D^{13°}$ -158°. This compound yielded a crystalline diacetate, m.p. 158°/

158° , $[\alpha]_D^{15°}$ -151°, and a ditosyl derivative which suffered no reaction on heating with sodium iodide in acetone. These facts together with the isolation of a crystalline monoacetone derivative makes it possible to propose with certainty structure (XX).



(XX)

This result and that obtained in the galactose series demonstrate the probability that the parent osazones may be represented by ketopyranose structures as in the case of glucosazone and galactosazone. The introduction of the methyl group in the hydrazide residue has therefore no effect on the ring structure of the osazone in the sense of imposing an acyclic structure as found in galactose-phenylmethylhydrazone (21). If (A) and (B) possess the β-configuration (XXI) then

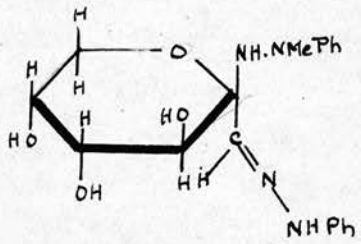


(XXI)

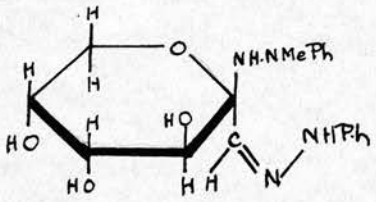
inversion has taken place on C_3 during anhydride formation/

formation and (XX) is a derivative of allose.

The structure of the isomeric glucosephenyl-phenylmethylosazones (A) and (B) remains for discussion. The specific rotations of (A) and (B) would seem to be too similar for the differences between them to be accounted for by a cyclic structure in one case and an acyclic one in the other, and the fact that both yield an anhydride of the above structure suggests that both are fructopyranosazones. It is possible that the differences may be accounted for on the basis that one is a syn- (XXII) and the other an anti- (XXIII) form. Another alternative is that one is the isomeric azo-form, $-CH_2-N=NPh$ as suggested by Zerner and Waltuch (24) although rearrangement would be necessary in this case to supply the hydrogen atom for anhydride formation.



(XXII)



(XXIII)

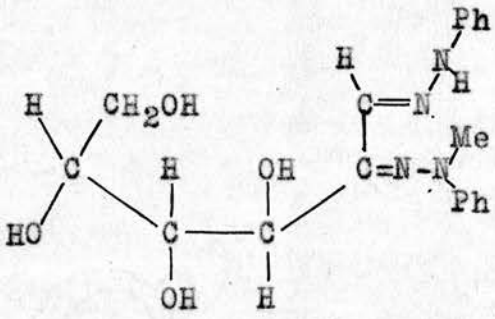
It may be demonstrated by means of models that in the case of (XXII) anhydride formation on C_3 is impossible and it would be necessary to suppose that during/

during the acetylation which precedes anhydride formation the syn-form is converted to the anti-form or an equilibrium mixture of the two is produced; this is supported by the observation that the acetates formed from (A) and (B) are amorphous and indistinguishable, although (A) and (B) themselves are quite distinct from one another. It must be pointed out that the yield of anhydro-compound is not quantitative; this may be due to the fact that the acetates are mixtures of α- and β-forms, the β-form being unable to form the anhydride unless inversion occurs at C_3. The experimental results, however, do not enable us to decide between any of these hypothetical structures.

As suggested previously (22) it is probable that the fructosephenylmethylhydrazones differ because one, ($[\alpha]_D^{17°}$ -253°) is cyclic and the other is acyclic. From the above results osazone (A) is derived from the cyclic and (B) from the acyclic form although a pyranose ring then appears in (B). Why different osazones finally result is not clear, although it may be permitted to speculate that in the acyclic case the entering phenylhydrazine residue is forced into the syn-form (XXIV) by the repulsive effect

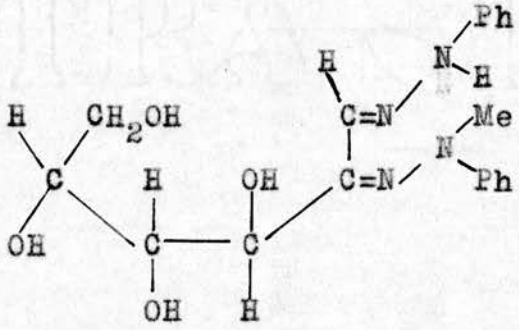
of/

of the C=N.NMePh which is rigidly attached by the double bond in a plane perpendicular to that containing the carbon atoms; this process being followed by



(XXIV)

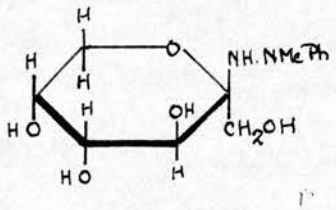
ring formation to give (XXII). If, indeed the existing phenylmethylhydrazine residue were also of the syn-type the entering group would be forced to take up the syn-form (XXV) for reasons of space.



(XXV)

If this is the case (B) is (XXII) the syn- and (A) is (XXIII) the anti-form, for the same considerations would not apply to the formation of an osazone from the cyclic fructosephenylmethylhydrazone (XXVI) provided the ring is not broken on the oxidation of $-CH_2OH$ to -CHO, owing to the absence of the rigidity/

rigidity conferred by the >C=N-NMePh group at C_2; it is not clear, however, why the anti-form should be preferred in this case.



(XXVI)

If the production of an azo-form is concerned a similar explanation could be advanced, for the transference of the double bond from >C=N- to -N=N- would also relieve congestion in the same way.

E X P E R I M E N T A L

Galactosephenylmethyl-phenylosazone and its Tetra-acetate

Galactosephenylmethylhydrazone (5 g.), m.p. 182°, $[\alpha]_D^{18°}$ +5° in pyridine-alcohol (3:2; c, 0.4), in alcohol (150 c.c.) was treated with phenylhydrazine (5 g.) and acetic acid (3 c.c.) at 100° for 20 hours. The product (6 g.) was isolated by the addition of water and cooling and had m.p. 178°, $[\alpha]_D^{17°}$ +98° in pyridine-alcohol (1:1; c, 0.4), +71° (21 hours); +62° (45 hours); +45° (100 hours, constant).

Found: C, 60.8; H, 6.6; N, 15.2.

$C_{19}H_{24}O_4N_4$ requires

C, 61.3; H, 6.5; N, 15.05%.

The osazone (5 g.) was dissolved in a mixture of acetic anhydride (10 c.c.) and pyridine (15 c.c.), and the product poured into water after 2 days and recrystallised from alcohol; it had m.p. 183°, $[\alpha]_D^{13°}$ +85° in chloroform (c, 0.4).

Found: C, 60.1; H, 6.0; N, 10.5; $CH_3.CO$, 32.8.

$C_{27}H_{32}O_8N_4$ requires

C, 60.0; H, 6.0; N, 10.4; CH .CO, 31.8%

Anhydrogalactosephenylmethyl-phenylosazone its Diacetate and Di-p-toluenesulphonate

The tetra-acetate (6.5 g.) in acetone (500 c.c.) was treated with 8% sodium hydroxide solution (80 c.c.) and water (300 c.c.). After 1 day the product (3 g.) was collected and recrystallised from acetone-light petroleum; it had m.p. 172°, $[\alpha]_D^{13°}$ +100° in acetone (c, 0.4).

Found: C, 65.0; H, 6.2; N, 15.4.

$C_{19}H_{22}O_3N_4$ requires

C, 64.4; H, 6.3; N, 15.8%.

The diacetate was obtained on acetylation as on p27 and recrystallised from alcohol, forming pale yellow needles, m.p. 170°, $[\alpha]_D^{14°}$ +50° in chloroform (c, 0.4)

Found: C, 62.3; H, 5.8; N, 13.1; $CH_3.CO$, 19.6.

$C_{23}H_{26}O_5N_4$ requires

C, 63.0; H, 6.1; N, 12.8; $CH_3.CO$, 19.6%

The anhydride (0.5 g.) was treated with p-toluenesulphonyl chloride (1 g.) in pyridine (4 c.c.) for 2 days. On pouring into water, a yellow powder was obtained, which separated from aqueous alcohol as a yellow microcrystalline powder, m.p. 65-70°(decomp), $[\alpha]_D^{15°}$ +37° in chloroform (c, 0.5).

Found: C, 60.5; H, 5.3; N, 8.55; S, 8.9.

$C_{33}H_{34}O_7N_4S_2$ requires/

$C_{33}H_{34}O_7N_4S_2$ requires
C, 59.8; H, 5.2; N, 8.5; S, 9.7%.

Ditosylanhydrogalactosephenylmethyl-phenylosazone (1 g.) was heated for 20 hours at 100° with sodium iodide (1.5 g.) in acetone (7.5 c.c.). Treatment with water yielded a brown solid (0.4 g.) devoid of iodine but containing nitrogen and sulphur. When this treatment was repeated for 7 hours on another specimen, the product was again devoid of iodine and appeared to be an impure monotosyl ester Found: N, 11.4. $C_{26}H_{28}O_5N_4S$ requires N, 11.2%

In attempts to condense the anhydride with acetone as described below in the glucose series by shaking with anhydrous copper sulphate the anhydride was recovered unchanged.

Galactosephenylhydrazone and Phenylmethylhydrazine

Galactosephenylhydrazone (10 g.) was heated for 2.5 hours with phenylmethylhydrazine (10 g.) and acetic acid (5.5 c.c.) in alcohol (600 c.c.). Galactosephenylmethylhydrazone (7 g.) rapidly formed. In another experiment the heating was continued for 8 hours to yield galactosephenylmethylhydrazone (7.5 g.) and an osazone (1.2 g.), m.p. 175°, $[\alpha]_D^{15°}$ +96° in pyridine-alcohol (1:1; c, 0.45), falling

to/

to +65° in 47 hours. This proved to be the same osazone as described on p27, since it yielded an acetate, m.p. 180°, $[\alpha]_D^{15°}$ +86° in chloroform (c, 0.5), which on deacetylation yielded an anhydride, m.p. 171-172°, unchanged on admixture with the anhydride described on p.28, $[\alpha]_D^{15°}$ +98° in acetone (c, 0.3).

Glucosephenylhydrazone and Phenylmethylhydrazine

Glucosephenylhydrazone (12 g.), phenylmethylhydrazine (10 g.), acetic acid (5 c.c.), water (450 c.c.), and a little sodium bisulphite were heated at 95-100°. After 45 minutes a crop of crystals (1) (3.5 g.), m.p. 193-195°, was removed, and after a further hour crop (2) (1.2 g.), m.p. 180°, was isolated. Three further crops (2.9 g.) similar to (2) were obtained. On extraction of (1) with hot alcohol a solution was obtained from which an osazone (A) crystallised on cooling (2.2 g.), m.p. 184°, $[\alpha]_D^{15°}$ -53° in pyridine-alcohol (1:1; c, 0.5); -19° (19 hours); -6° (68 hours, constant). The residue (B) (1.3 g.) had m.p. 202-203°, $[\alpha]_D^{15°}$ -60° in pyridine-alcohol (1:1; c, 0.4), -20° (24 hours); -15° (40 hours, constant). Mixed m.p. of (A) and (B)/

(B), 183°.

Found: (A) C, 60.7; H, 6.5; N, 15.4.

(B) C, 60.8; H, 6.5; N, 15.3.

Calc. for $C_{19}H_{24}O_4N_4$:

C, 61.3; H, 6.5; N, 15.05%.

Fraction (2) on recrystallisation gave an osazone, m.p. 192-194°, identical with (A), as did also the remaining fractions.

Glucosephenylmethylhydrazone and Phenylhydrazine

Glucosephenylmethylhydrazone (18 g.), m.p. 132°, $[\alpha]_D^{19°}$ +5° in water (c, 1.6), was heated with water (250 c.c.), phenylhydrazine (13.5 g.), acetic acid (7.5 c.c.), and sodium bisulphite. Five fractions of osazone (11 g.) were separated, the first after heating for 30 minutes. This had m.p. 200°, raised to 202° [unchanged on admixture with (B)] on recrystallisation, $[\alpha]_D^{15°}$ -62° in pyridine-alcohol (1:1; c, 0.5), -30° (12 hours), -14° (43 hours, constant). All the other fractions had similar properties.

In a second experiment half the above proportion of phenylhydrazine was used and six fractions were isolated. Fraction (1) had m.p. 201-202°, $[\alpha]_D^{15°}$ -62° in pyridine-alcohol (1:1; c, 0.4), -22° (21 hours), -16° (45 hours, constant). The other five fractions/

fractions were similar and all gave the same anhydride on acetylation and subsequent deacetylation.

In a third experiment glucosephenylmethylhydrazone (20 g.) in alcohol (500 c.c.) was heated for 8 hours with phenylhydrazine (25 g.) and acetic acid (13 c.c.) to yield an osazone (14 g.), m.p. 205°, $[\alpha]_D^{15°}$ -70° in pyridine-alcohol (1:1; c, 0.5), -29° (17 hours, constant). Acetylation and deacetylation gave the dianhydrohexosazone, m.p. 235°, previously described (5), confirmed by the isolation of the monoacetate, m.p. 135°, and comparison with authentic specimens.

Glucosephenyl-phenylmethylosazone Anhydride

Osazones (A) and (B) were acetylated in the usual way to yield amorphous acetates. Acetate (A) had $[\alpha]_D^{13°}$ -44° in chloroform (c, 0.4)
Found: C, 59.8; H, 5.7; N, 10.7; $CH_3.CO$, 32.7.
$C_{27}H_{32}O_8N_4$ requires
C, 60.0; H, 6.0; N, 10.4; $CH_3.CO$, 31.8%.
and acetate (B), $[\alpha]_D^{15°}$ -43° in chloroform (c, 0.5).
Found: N, 10.6%.

Acetate (A) (1.5 g.) was deacetylated as described for the galactose derivative to yield a crystalline anhydride (0.7 g.), obtained from acetone-light petroleum in lemon-yellow needles, m.p. 176-178°, $[\alpha]_D^{13°}$ -158° in acetone (c, 0.4).
Found/

Found: C, 64.3; H, 6.3; N, 15.9.

$C_{19}H_{22}O_3N_4$ requires

C, 64.4; H, 6.3; N, 15.8%.

Acetate (B) on similar treatment gave the same glucosephenyl-phenylmethylosazone anhydride, m.p.176° $[\alpha]_D^{15°}$ -155° in acetone (c, 0.5); a mixed m.p. with the anhydride from (A) showed no depression.

Anhydroglucosephenyl-phenylmethyl-osazone Diacetate

By acetylation as before a product was obtained in quantitative yield which, after recrystallisation from alcohol, had m.p. 158°, $[\alpha]_D^{15°}$ -151° in chloroform (c, 0.5), and gave the original anhydride, m.p. 177°, on deacetylation.

Found: C, 62.9; H, 6.0; N, 12.4; $CH_3.CO$, 19.7.

$C_{23}H_{26}O_5N_4$ requires

C, 63.0; H, 6.1; N, 12.8; $CH_3.CO$, 19.6%.

Acetone Anhydroglucosephenyl-phenylmethylosazone

The anhydride (0.2 g.) was shaken with acetone (100 c.c.) and anhydrous copper sulphate (20 g.) for 3 days. After filtration and evaporation a product was obtained (0.25 g.) which on recrystallisation from acetone-light petroleum had m.p. 160°, $[\alpha]_D^{18°}$ -33°/

-33° in acetone (c, 0.5).

Found: C, 67.1; H, 6.7; N, 14.5.

$C_{22}H_{25}O_3N_4$ requires

C, 67.0; H, 6.6; N, 14.2%.

Ditosyl Anhydroglucosephenyl phenylmethylosazone

This compound, prepared as in the galactose series and isolated as a yellow powder, had m.p. 65-70° (decomp.), $[\alpha]_D^{15°}$ -80° in chloroform (c, 0.4)

Found: C, 60.0; H, 5.2; N, 8.6.

$C_{33}H_{34}O_7N_4S_2$ requires

C, 59.8; H, 5.2; N, 8.5%.

This product was treated with sodium iodide in acetone at 100° for 14 hours; the resultant brownish-yellow material contained nitrogen and sulphur but was devoid of iodine.

Fructosephenylmethylhydrazones and Phenylhydrazine

(1) Ofner's phenylmethylhydrazone, m.p. 116°.

This substance, $[\alpha]_D^{15°}$-7° in pyridine-alcohol (1:1; c, 0.9) (6.1 g.) in water (84 c.c.) was heated at 95-100° with phenylhydrazine (4.5 g.), acetic acid (2.4 c.c.), and sodium bisulphite; crystals appeared after/

after 1 minute (in 0.6% solution no osazone appeared after 45 minutes until the solution was cooled). Five crops of osazone (5.6 g.) were obtained with m.p.'s varying between 203-204°, $[\alpha]_D^{18°}$ -61° in pyridine-alcohol (1:1; c, 0.5), -20° (18 hours), -14° (43 hours, constant). Admixture with osazone (B) from glucose-phenyl- or -phenylmethylhydrazone did not depress the m.p.

Found: C, 60.9; H, 6.4; N, 14.9.

Calc. for $C_{19}H_{24}O_4N_4$:

C, 61.3; H, 6.5; N, 15.05%.

The first four fractions were acetylated to yield identical acetates, $[\alpha]_D^{18°}$ -43° in chloroform (c, 0.5),

Found: C, 59.9; H, 6.0; N, 10.6; $CH_3.CO$, 32.9.

Calc. for $C_{27}H_{32}O_8N_4$:

C, 60.0; H, 6.0; N, 10.4; $CH_3.CO$, 31.8%,

which were deacetylated as before. In every case a product was obtained in good yield which, on recrystallisation from acetone-light petroleum yielded the anhydride described on p. 33, m.p. 176°, $[\alpha]_D^{16°}$ -155° in acetone (c, 0.5), confirmed by determinations of mixed m.p. This result was twice confirmed.

(2) Fructosephenylmethylhydrazone, m.p. 170°.

Osazone formation in the usual way yielded pale/

pale yellow needles, m.p. 194-195°, not depressed by osazone (A) from glucosephenylhydrazone, $[\alpha]_D^{15°}$ -54° in pyridine-alcohol (1:1; c, 0.5), -23° (18 hours), -7° (44 hours, constant). The osazone was formed in less than 1 minute from a 0.6% solution of the hydrazone.

Found: C, 60.7; H, 6.5; N, 15.3.

Calc. for $C_{19}H_{24}O_4N_4$:

C, 61.3; H, 6.5; N, 15.05%.

Acetylation yielded quantitatively a tetra-acetate, $[\alpha]_D^{13°}$ -44° in chloroform (c, 0.4)

Found: C, 59.8; H, 5.9; N, 10.7; $CH_3.CO$, 33.0.

Calc. for $C_{27}H_{32}O_8N_4$:

C, 60.0; H, 6.0; N, 10.4; $CH_3.CO$, 31.8%.

Deacetylation of this acetate (2.2.g.) in acetone (150 c.c.) with 0.5N-sodium hydroxide (112 c.c.) yielded the anhydride previously described (1.1 g.), m.p. 176-177, $[\alpha]_D^{15°}$ -158° in acetone (c, 0.4)

Found: C, 64.3; H, 6.3; N, 15.5.

Calc. for $C_{19}H_{22}O_3N_4$:

C, 64.4; H, 6.3; N, 15.8%.

Acetylation yielded the diacetate, m.p. 157-158°, $[\alpha]_D^{15°}$ -151° in chloroform (c, 0.6).

Found: $CH_3.CO$, 19.6. Calc. for $C_{23}H_{26}O_5N_4$: $CH_3.CO$, 19.6%.

S_U_M_M_A_R_Y

1. The preparation of galactosephenylmethyl-phenylosazone and of its anhydride is described.

2. A structure is proposed for the latter including a 2:6-oxide ring and a 2:4-pyrazoline ring on the basis of the failure of the product to condense with acetone, the formation of a diacetate and a di-p-toluenesulphonate and the fact that the latter compound undergoes no reaction with sodium iodide in acetone.

3. It is concluded that inversion does not take place at C_2 during anhydride formation.

4. The two isomeric glucosephenylmethyl-phenylosazones of Votoček and Vondráček give rise to the same monoanhydride.

5. A structure is proposed for this compound embodying a 2:6-oxide ring and a 1:3-pyrazoline ring since it forms a monoacetone derivative, a diacetate and a di-p-toluenesulphonate which did not react with sodium iodide in acetone.

6. It is pointed out that the presence of the phenylmethylhydrazine residue appears to have no effect on the ketopyranose character of the osazone anhydrides and probably of the mixed osazones themselves.

7. Speculations are made as to the reasons for the differences between the two isomeric fructose-phenylmethyl-phenylosazones. It is made clear that the suggestion of Votoček and Vondráček that one is glucosephenylmethyl-phenylosazone and the other glucosephenyl-phenylmethylosazone is incorrect. It is suggested that the differences are due either to geometric isomerism, or to the fact that one contains an azo-group.

8. The bearing of the above facts on the constitution of the two isomeric fructosephenylmethylhydrazones is discueesed and it is considered that the acyclic hydrazone is more likely to yield a <u>syn</u>-form (or an azo-form) in the resulting osazone because of the fact that the double bond confers a rigidity of structure which, if ring formation is subsequent to the entry of the second phenylhydrazine residue into the molecule, will/

will cause that entering group to take up a position as remote as possible from the phenylmethylhydrazine residue.

B_I_B_L_I_O_G_R_A_P_H_Y

1.	Haworth ----------	The Constitution of Sugars, 1929, Ed. Arnold and Co.
2.	Emil Fischer ----------	Ber., 1887, 20, 830.
3.	E.E.Percival and E.G.V.Percival-	J., 1935, 1398.
4.	L.v.Vargha ----------	Ber., 1936, 69, 2102.
5.	E.G.V.Percival ----------	J., 1936, 1771.
6.	Haworth,Hirst and Learner -----	J., 1927, 1040.
7.	Engel ----------	J.A.C.S., 1935,57,2419.
8.	Muskat ----------	J.A.C.S., 1934,693;2449.
9.	Maurer and Schiedt ----------	Ber., 1935, 68, 2187.
10.	Wolfrom,Konigsberg and Soltzberg	J.A.C.S.,1936,58,490.
11.	Kunz and Hudson ----------	J.A.C.S.,1926,48,1982.
12.	Behrend and Reinsberg ---------	Annalen,1910,377,187.
13.	E.E.Percival and E.G.V.Percival -	J., 1937, 1320.
14.	Diels,Cluss,Stephan and König -	Ber., 1938, 71, 1198.
15.	E.G.V.Percival ----------	J., 1938, 1384.
16.	Oldham and Rutherford ---------	J.A.C.S.,1932,54,366.
17.	Diels and Meyer ----------	Annalen,1935,519,157.
18.	Diels,Meyer and Onnen ---------	Annalen,1936,525, 94.
19.	Muir and E.G.V.Percival -------	J., 1940, 1479.
20.	Wolfrom and Christman ---------	J.A.C.S.,1931,53,3413.
21.	Ofner ----------	Monatsh.,1905,26,1165.
22.	Percival and Heddle ----------	J., 1940, 1511.
23.	Votocek and Vondracek----------	Ber., 1904,37, 3848.
24.	Zerner and Waltuch ----------	Monatsh.,1914,35,1025.

P_A_R_T_ II

A study of Sugar Phenylhydrazones

Investigations on Sugar Phenylhydrazones

It is usually the convention to represent the hydrazones of sugars as straight chain compounds, but there would seem to be no reason why this should be so. Undoubtedly galactosephenylmethylhydrazone is acyclic as proved by Wolfrom and Christman (1) who converted aldehydo glucose pentaacetate into penta-acetyl glucosephenylmethylhydrazone and also obtained an identical compound from the mild acetylation of glucosephenylmethylhydrazone. On the other hand of the two fructosephenylmethylhydrazones mentioned in the preceding section (p. 24) it is thought probable that one has a cyclic structure.

In 1908 Behrend and Lohr (2) suggested that the two known glucosephenylhydrazones represented in one case (so-called dextrose-β-phenylhydrazone, m.p. 141°) a true acyclic hydrazone and the α-form, m.p. 160° was a hydrazide:

$$CH_2(OH).CH(OH).\overbrace{CH.[CH(OH)]_2.CH}^{O}.NH.NHPh$$

Two years later Behrend and Reinsberg (3) following up the acetylation experiments of Hofmann (4) showed that dextrose-β-phenylhydrazone gave an amorphous acetate/

acetate whilst dextrose-α-phenylhydrazone gave a crystalline acetate together with an amorphous acetate. These compounds are shown to be pentaacetates. True hydrazones yield N-acetyl derivatives with difficulty, and since the crystalline dextrose-α-phenylhydrazone pentaacetate yields acetyl phenylbenzylidenehydrazone on treatment with potassium hydroxide and benzaldehyde whereas the amorphous dextrose-β-phenylhydrazone pentaacetate gave phenylbenzylidenehydrazone it was concluded that in the α-pentaacetate an acetyl group was attached to a nitrogen atom and was therefore produced from a hydrazide, whereas in the β-pentaacetate no such N-acetyl group existed. Further the dextrose-α-phenylhydrazone pentaacetate gave α-acetylphenylhydrazine on treatment with hydrochloric acid. Confirmation was obtained by condensing glucose with α-acetylphenylhydrazine followed by acetylation to yield the previously described glucose-α-phenylhydrazone pentaacetate.

In 1908 Irvine and Moodie (5) attempted the methylation of tetramethyl glucosephenylhydrazone with silver oxide and methyl iodide. They suggest that hydrolysis showed that a methyl group was introduced into the γ-position of the sugar chain and also into/

into the phenylhydrazine residue, but the difficulties encountered in the attempted hydrolysis with aqueous methyl-alcoholic-hydrogen chloride make this conclusion of doubtful value.

It was decided to reinvestigate this problem, and glucose-α-phenylhydrazone was prepared according to Stempel (6). It had m.p. 159°, $[\alpha]_D^{15°}$ -82°. This value fell to -12° in 60 hours and the solution became reducing showing that decomposition had occurred. On the other hand the high numerical initial rotation would not be inconsistent with the presence of a ring structure. Methylation experiments were therefore instituted although it was feared that the substance might be too unstable on account of its behaviour in aqueous solution. By two methylations with methyl sulphate and sodium hydroxide a product of OMe, 30.8% was obrained which by further treatment with Purdie's reagents was raised to a maximum of 34.6%. This result was confirmed and the product a golden mobile syrup had $n_D^{16°}$ 1.5430, $[\alpha]_D^{15°}$ +29.5° in methyl alcohol (c, 0.5); the methoxyl and methylimino content of which was fairly close to that of tetramethyl glucosephenylmethylhydrazone. If the glucosephenylhydrazone had been acyclic the resulting pentamethyl glucosephenylmethylhydrazone/

methylhydrazone would have had OMe, 43.9%. Thus it would appear that a cyclic structure is present, although since there is loss of yield during methylation the destruction in solution of an acyclic form is not precluded. One would have expected then however to have prepared tetramethyl methylglucoside (OMe, 62%) and although distillation in a high vacuum was attempted this compound could not be isolated. As a matter of interest the product was compared with tetramethyl glucopyranosephenylmethylhydrazone (prepared from tetramethyl glucose), which was obtained as a golden syrup, $[\alpha]_D$ +39° (in methyl alcohol), n_D 1.5290: the similarity is obvious and it is unfortunate that neither product could be induced to crystallise.

Difficulties were met with in attempts to remove the phenylmethylhydrazine residue with hydrochloric acid or p-nitrobenzaldehyde so that it proved impossible to determine the nature of the oxide ring apparently present. The authentic tetramethyl glucosephenylmethylhydrazone proved just as intractable and it may be recalled that Purdie and Irvine (7) showed that the corresponding phenylhydrazone was not resolved into its components with benzaldehyde or fum-

ing/

ing hydrochloric acid.

Glucosephenylmethylhydrazone was next studied. This substance had been described by Ofner (8) and by Neuberg (9). Owing to its insoluble character it was found impracticable to determine the optical rotation but the pentaacetate which was described for the first time, m.p. 113-114°, had a very high specific rotation, +157° in chloroform. Two methylations gave a product, OMe, 28.6%. Further methylation failed to raise the value above 33%.

E_X_P_E_R_I_M_E_N_T_A_L

Preparation of Glucosephenylhydrazone

d-Glucose (40 g.) dissolved in a mixture of acetic acid (30 g.) and water (10 g.) was converted into the glucosephenylhydrazone according to the method of Stempel (6). A white solid was obtained which was filtered and washed with ether, m.p. 142° No further precipitate was obtained on allowing the filtrate to stand for a further period in ice. The crude product was recrystallised from hot alcohol; m.p. 151-2° after one recrystallisation. (Yield 26 g.). A second preparation from 40 g. glucose gave 38 g. glucosephenylhydrazone.

Purification.- The crude product was boiled in acetone and filtered while hot to yield white shining flat needles, m.p. 159°, $[\alpha]_D^{18°}$ -82° in water. On allowing the acetone filtrate to stand rosettes of white needles came down, m.p. 110°. On further standing a white powder sometimes settled m.p. 145°.

Mutarotation.- Glucosephenylhydrazone (0.1030 g.) in water (10 c.c.) gave $[\alpha]_D^{18°}$ -82° (2 mins.); -78° (4 mins.); -76° (5 mins.); -62° (12 mins.); -55° (24 mins.); -53° (29 mins.); -33° (14.5 hours); -26° (24 hours); -20° (36 hours); -12° (60 hours, constant)

On/

On allowing the rotation solution to stand it turned brown and was found to be reducing to Fehling's solution.

Methylation of Glucosephenylhydrazone

Glucosephenylhydrazone (20 g.) in water (50 c.c.) and acetone (50 c.c.) was methylated in the usual manner with methyl sulphate (60 c.c.) and sodium hydroxide (170 c.c.) at 45°. A further quantity of acetone (50 c.c.) was added after half an hour. After the final addition of the reagents the temperature was raised to 75° for 10 minutes. A brown tarry mass was thrown down on cooling. This was filtered, dissolved in chloroform, the chloroform solution washed with water till neutral and dried with sodium sulphate, and the solvent removed under reduced pressure to give a brown syrup (OMe, 26.4%).

The original filtrate from the methylation was extracted with chloroform, the extract washed with water, dried with sodium sulphate, and the solvent removed under diminished pressure, to yield a brown syrup (OMe, 21%).

Remethylation.- The above syrup (OMe, 26.4%) was dissolved in acetone (100 c.c.) and water (100 c.c.) and remethylated as above. A brown oil was deposited. A further quantity of water was added and the mixture filtered through cotton wool. The brown oil

and/

and the cotton wool were extracted with chloroform and the chloroform solution treated as above to yield a non-reducing golden brown tar (OMe, 30.8%).

Remethylation according to Purdie.- The above tar (OMe, 30.8%) was methylated with methyl iodide (100 g.) and silver oxide (30 g.) and the mixture allowed to stand overnight. The product was filtered and the precipitate extracted several times with boiling ether. The filtrate and ethereal extracts were mixed and taken to dryness to yield a brown tar (OMe, 33.4%).

This was subjected to a further Purdie methylation and treated as above to yield a brown tar (OMe, 34.3%).

Purification.- The above tar was purified by extraction with chloroform, washing the extract with acetic acid and then with water till neutral and evaporation to dryness. The resulting syrup was dissolved in a minimum quantity of chloroform and a correspondingly large quantity of light petroleum (b.p. 40-60°) added. A dark coloured oil was deposited and allowed to settle. The clear solution was decanted and taken to dryness to yield an orange red tar (OMe, 34.6%). The dark oil deposited above was dried to yield a dark tar (OMe, 31.3%).

Methylation of Glucosephenylhydrazone (II)

A second batch of glucosephenylhydrazone (25 g.) was subjected to four methylations with methyl sulphate as in the first case and the product purified. The final chloroform solution was washed with sulphuric acid and the resulting syrup dissolved twice in light petroleum (b.p. 40-60°) (1 litre). A mobile syrup was obtained (OMe, 28.8%).

Further Purification.- The syrup was dissolved in the minimum quantity of chloroform and impurity precipitated with light petroleum (b.p. 40-60°) (1 litre). The clear solution was decanted through cotton wool and the solvent removed. This was repeated six times to yield a mobile golden yellow syrup. (8.5 g.). Found: OMe, 32.4%; NMe, 7.4%.

This syrup (OMe, 32.4%) was methylated twice according to Purdie and the resulting syrup dissolved in the minimum quantity of chloroform. On addition of light petroleum (b.p. 40-60°) a dark oil was deposited. The clear solution was decanted and evaporated to dryness. On repeating this only a negligible quantity of dark oil was deposited. The petroleum solution gave a mobile oil (OMe, 32.8%).

This oil was subjected to three further Purdie methylations/

methylations and the final product was subjected to four purifications as above to yield a golden red mobile syrup, n_D 1.5430, $[\alpha]_D^{18°}$ +29.5°.

Found: OMe, 34.6; NMe, 7.5.

Calc. for tetramethyl glucosephenylmethylhydrazone, $C_{17}H_{28}N_2O_5$, OMe, 36.4; NMe, 8.5%.

The syrup was moderately soluble in water and gave a positive carbylamine test. Addition of sodium hydroxide and chloroform gave a product strongly reducing to Fehling's solution.

Attempted Fractionation.- The above syrup was subjected to distillation in high vacuum, but decomposition appeared to take place: Fraction I was a colourless highly smelling syrup; Fraction II was a pale yellow oil, OMe, 33.2%.

Preparation of Tetramethyl Glucosephenylmethylhydrazone starting with Tetramethyl Glucose

Tetramethyl glucose (1 g.) was dissolved in water (1 g.) and phenylmethylhydrazine (0.5 g.) added. (5). Acetic acid (2 drops) was added until the emulsion cleared and the mixture was allowed to stand 48 hours. An oil was deposited. The mixture was diluted with water and filtered. The oil was dissolved in chloroform and the chloroform solution acidified with acetic acid, washed with water, dried with anhydrous sodium sulphate and taken to dryness to yield a non-

reducing/

reducing golden yellow syrup (1 g.), $[\alpha]_D^{17°}$ +39° in methyl alcohol (c, 0.45), $n_D^{17°}$ 1.5290.

Attempted Hydrolysis of Methylated Glucose phenylhydrazone with p-Nitrobenzaldehyde

Methylated glucosephenylhydrazone (OMe, 34.6%, 0.25 g.) was dissolved in hot alcohol (7 c.c.) and p-nitrobenzaldehyde (2.5 g.) added. This gave a dark red solution which on the addition of water (7 c.c.) gave rise to a dark red oil. Alcohol (6 c.c.) was added and the mixture heated on a water-bath for half an hour. A brown oil was deposited which on cooling changed to a yellowish red solid, which was non-reducing. A further quantity of alcohol (12 c.c.) was added and the mixture heated under reflux for 18 hours and filtered. The alcohol was removed by evaporation and the solution on dilution with water was found to be non-reducing to Fehling's solution.

This hydrolysis was repeated on a fresh sample of methylated glucosephenylhydrazone and the conditions modified, but the final product was again non-reducing to Fehling's solution.

Attempted Hydrolysis of Methylated Glucosephenylhydrazone with Concentrated Hydrochloric Acid

After unsuccessful attempts to hydrolyse the methylated material with oxalic acid and dilute hydrochloric acid, both of which gave rise to non-reducing/

ducing syrups, preliminary attempts were carried out with concentrated hydrochloric acid. This was found to yield a syrup which was slightly reducing to Fehling's solution. Further experiments were therefore instituted in order to ascertain the best conditions for hydrolysis with this reagent.

Methylated glucosephenylhydrazone (OMe, 34.6%, 0.5 g.) was treated with concentrated hydrochloric acid (2.5 c.c.) and the reducing power of samples withdrawn at intervals of 1, 2, 5, 10, 15, 20, 30 minutes, 1 hour and 16 hours, tested. The reducing power was found to be strongest after 30 minutes; longer periods showed a decrease in reducing action, and after 16 hours the sample was practically non-reducing.

Typical Attempted Hydrolysis of Methylated Glucose-phenylhydrazone with Concentrated Hydrochloric Acid for Thirty Minutes

The methylated glucosephenylhydrazone (OMe, 34.6% 3 g.) was hydrolysed with concentrated hydrochloric acid (10 c.c.) for 30 minutes. The mixture was then diluted with water and the precipitate that was deposited was removed by filtration. The aqueous solution (A) was extracted five times with chloroform. The strongly reducing chloroform extracts were washed with water and sodium bicarbonate until they/

they were neutral, dried and the chloroform removed under reduced pressure to yield a golden brown syrup (1.5 g.). This syrup on treatment with water at 100° for 1 hour partly dissolved leaving behind a brown viscous tar (0.5 g.). The aqueous solution was decanted, acidified with dilute hydrochloric acid, and extracted with chloroform. The acidic residual solution (B) was investigated later. The chloroform extracts were neutralised with sodium bicarbonate, dried and the solvent removed to yield a golden yellow syrup. This syrup was extracted several times with light petroleum (b.p. 60-80°) and the solvent removed by evaporation. This was followed by extraction with ether, removal of the solvent and further extraction with light petroleum (b.p. 60-80°). In each case a small residue of non-reducing syrup remained undissolved. Final removal of the solvent gave a golden brown, slightly reducing syrup (0.3 g.), OMe, 39.5%, $[\alpha]_D^{17°}$ +37° in methyl alcohol (c, 1.0).

Attempted Conversion to the Glycoside.- The above syrup (0.3 g.) was treated with 2% methyl-alcoholic hydrogen chloride (32 c.c.) under reflux for 13.5 hours at 50°, followed by 2.5 hours at 75°. The product was neutralised with silver carbonate, decolourised with charcoal, filtered and the precipitate/

tate washed with ether. The filtrate and washings were evaporated to dryness and the resulting syrup distilled in a high vacuum to yield 2 fractions.
Fraction I. b.p. 100°/0.07 mm. pale yellow mobile syrup. $n_D^{17°}$ 1.4520. OMe, 52.0%. 0.2 g.
Fraction II. b.p. 120-160°/0.07 mm. colourless syrup $n_D^{17°}$ 1.5370. 0.05 g.

Attempted Hydrolysis to the Methylated Sugar.- Fraction I (0.2 g.) was hydrolysed with 5% hydrochloric acid (7 c.c.) at 95° for 7.5 hours. The mixture was neutralised with barium carbonate, filtered, the residue washed with alcohol and the aqueous alcoholic solution and washings evaporated to dryness. Extraction with ether, followed by light petroleum (b.p. 60-80°) and removal of the solvent gave a syrup, (0.15 g.), which on standing made tentative attempts to crystallise. (OMe, 41.8%) $[\alpha]_D^{17°}$ +15° in chloroform (c, 0.5).

Attempted Conversion to the Anilide.- The mixture of syrup and crystals (0.1 g.) was heated with alcohol (1 c.c.) and aniline (0.2 c.c.) for 4 hours. Excess of aniline was removed in a high vacuum and a mixture of tar and crystals was obtained. Repeated recrystallisation gave crystals (0.001 g.), m.p. 130° Authentic tetramethyl glucose anilide give m.p. 138°. The quantity of crystals was not sufficient for further investigation./

ther investigation.

The aqueous and acidic residual solutions (A) and (B) were combined and made alkaline with sodium hydroxide and extracted three times with chloroform. The combined chloroform extracts on investigation gave a dark brown syrup which had negligible methoxyl content. The alkaline residual solution was acidified with hydrochloric acid, neutralised with sodium bicarbonate and taken to dryness. The residue was extracted with boiling chloroform until the final residue in the flask was non-reducing and the combined extracts were evaporated to dryness to yield a yellow syrup (0.3 g.). This syrup was repeatedly extracted with ether under reflux . The ethereal extracts were evaporated to dryness to yield a syrup which showed tentative signs of crystallisation. (0.15 g.) (OMe, 14.1%). It was impossible to obtain the crystals free from syrup in sufficient quantity to prove their identity.

Several hydrolytic experiments on methylated glucosephenylhydrazone were carried out embodying various modifications of the above conditions, but they all failed to yield any satisfactory results.

Attempted Hydrolysis of Tetramethyl Glucose-phenylmethylhydrazone

Tetramethyl glucosephenylmethylhydrazone (1 g.) prepared/

prepared from tetramethyl glucose (page 50) was subjected to hydrolysis with concentrated hydrochloric acid (2.4 c.c.) during 30 minutes and the product worked up as in the previous case for methylated glucosephenylhydrazone.

The chloroform extracts gave a light brown syrup (0.5 g.), (OMe, 36%), $[\alpha]_D^{17°}$ +45° in methyl alcohol (c, 0.5).

Attempted conversion to the glycoside as in the previous case (page 53) gave a small quantity of a mobile yellow syrup (0.1 g.) (OMe, 48%).

The aqueous extracts, after similar treatment to that described on page 55, gave a syrup admixed with crystals (0.05 g.). It was impossible to separate and identify the crystals.

Preparation of Glucosephenylmethylhydrazone

Glucose (36 g.) was converted into glucosephenylmethylhydrazone according to the method of Ofner (8), modified by Neuberg (9). Glucosephenylmethylhydrazone was obtained as a white crystalline solid (20 g.) m.p. 131°.

Preparation of Glucosephenylmethylhydrazone Pentaacetate

Glucosephenylmethylhydrazone, (m.p. 131°; 3 g.) was acetylated by the addition of acetic anhydride (6 c.c.)/

(6 c.c.) and pyridine (16 c.c.) during 45 minutes with constant stirring; after 12 hours the mixture was poured into cold water. The white gummy solid obtained gave, on recrystallisation from alcohol, white shining prisms of the penta-acetate (2.5 g.), m.p. 113-114°, $[\alpha]_D$ +157° in chloroform (c, 0.5)

Found: $CH_3.CO$, 43.1; N, 5.9.

$C_{23}H_{30}O_{10}N_2$ requires

$CH_3.CO$, 43.6; N, 5.7%.

Attempted Methylation of Glucosephenyl methylhydrazone

Glucosephenylmethylhydrazone (5 g.) in water (30 c.c.) and acetone (30 c.c.) was methylated with sodium hydroxide (53 c.c., 30%) and methyl sulphate (20 c.c.), in one tenth portions every 10 minutes, at 45° until all the reagents were added. The temperature was raised to 85° and on cooling a brown syrup was deposited. Water was added and the whole mixture extracted several times with chloroform. The extracts were dried and the solvent removed to yield a slightly reducing light brown mobile syrup. (OMe, 27.5%).

This syrup was remethylated using the same conditions as before. An oil separated after 30 minutes and further quantities of acetone were added from time to time to bring the oil into solution.

A/

A light brown mobile non-reducing syrup was obtained. (2.5 g.). Found: OMe, 28.6. Calc. for trimethyl glucosephenylmethylhydrazone, $C_{16}H_{26}O_5N_2$, OMe, 28.5%.

Acetylation of Methylated Glucosephenyl methylhydrazone

The above syrup (2.5 g., OMe, 28.6%) was treated with acetic anhydride (3 c.c.) and pyridine (8 c.c.). After standing 12 hours the mixture was poured into large quantities of water and yielded a gummy solid, which was dissolved in chloroform, the chloroform solution washed, dried and taken to dryness to yield a brown viscid, non-reducing syrup, OMe, 21.2%.

Deacetylation and Methylation

The above syrup was methylated with sodium hydroxide and methyl sulphate in the usual way. Further quantities of acetone (100 c.c.) were added from time to time. After the final addition the mixture was heated to 65° for 5 minutes and cooled. A dark brown solid was deposited which was extracted twice with chloroform. These extracts after the usual treatment yielded a very viscid syrup. This syrup was subjected to a further methylation under the same conditions to yield a brown very viscid syrup (OMe, 33.0%). Tetramethyl glucosephenyl-methylhydrazone, $C_{17}H_{28}O_5N_2$, requires OMe, 36.4%.

Two further methylations according to Purdie failed to raise the methoxyl content.

S_U_M_M_A_R_Y

1. The methylation of glucose α-phenylhydrazone is shown to yield a tetramethyl glucosephenyl methylhydrazone indistinguishable from that prepared from tetramethylglucopyranose. This indicates a cyclic structure.

2. Evidence as to the type of oxide-ring by hydrolytic experiments could not be secured.

B_I_B_L_I_O_G_R_A_P_H_Y

1. Wolfrom and Christman --------J.A.C.S., 1931,53,3413.
2. Behrend and Lohr ------------Annalen,1908,362, 78.
3. Behrend and Reinsberg --------Annalen,1910,377, 189.
4. Hofmann ----------------------Annalen,1909,366, 306.
5. Irvine and Moodie ------------J., 1908, 93, 104.
6. Stempel ----------------------J.A.C.S., 1934, 1351.
7. Purdie and Irvine ------------J., 1903, 83, 1033.
8. Ofner ------------------------Monatsh., 1905,26,1165.
9. Neuberg ----------------------Ber., 1902, 35, 965.

P_A_R_T III

Investigation on a Polysaccharide Isolated from Chondrus Crispus

I_N_T_R_O_D_U_C_T_I_O_N

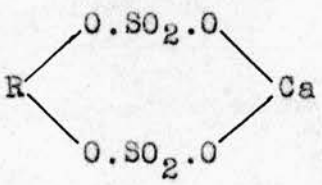
Carrageen or Irish moss finds an application as a thickener of jams and jellies, as a size and in pharmacy as an emulsifying agent.

In 1868 Flückiger and Obermayer (1) reported that treatment with nitric acid gave mucic acid and Bente (2) isolated laevulinic acid on heating the weed with mineral acid. Tollens and his school were however the first workers to make any kind of systematic attack on the subject. Haedicke, Bauer and Tollens (3) isolated 2 g. of galactose from 500 g. of weed and work by Müther and Tollens (4) indicated the presence of a small proportion of pentose or methyl pentose. Lintner, Düll and Kiermayer (5) considered that the isolation of hydroxymethylfurfuralphenylhydrazone indicated the presence of fructose, and Tollens(6) indeed placed carrageen among the fructosans. Sebor (7) designated Carrageen as a complex carbohydrate containing galactose, glucose and fructose residues together with a small quantity of pentose.

In more recent times attention has been paid to the problem by Haas and his co-workers. In 1921 Haas and Hill (8) showed that two distinct fractions could/

could be obtained by the extraction of Irish moss with water and Haas (9) published an account of a method of separating the two; one fraction being readily soluble in hot but sparingly soluble in cold water and the other readily soluble in both hot and cold water. It was shown that the two extracts had high ash contents, the cold extract (C.E.) having 21.6% and the hot extract (H.E.) 17.6%; these values were not diminished on prolonged dialysis.

From a systematic investigation of the H.E. the following facts were discovered. The ash consisted largely of calcium sulphate. The calcium was ionised in the polysaccharide since it was quantitatively precipitated from an aqueous solution of the H.E. by ammonium oxalate. On the other hand the sulphate group was not ionised and could not be precipitated by barium chloride until the carbohydrate had been hydrolysed with hydrochloric acid. The quantity of sulphate so obtained was approximately half that present in the ash. Haas therefore concluded that he was dealing with an ethereal sulphate which he formulated as



Such a formulation accounts for the loss of half the sulphate/

sulphate on ignition (as SO_3) and would explain the above facts. A comparison of the C.E. and H.E. was made by Russell-Wells (10) and an ethereal sulphate formula was found to apply to the C.E. also. The ratio of sulphate after hydrolysis to sulphate in the ash was greater than 2:1 which she explained as due to the presence of ammonium as well as calcium ion; in addition sodium, potassium and traces of iron and magnesium were reported. The same ions were also found in the H.E., but this contained more calcium and less sodium and potassium than the C.E. It was also stated that more mucic acid and less oxalic acid was obtained from the C.E. than the H.E., and pentose residues were indicated to be present in both extracts, again more in C.E.

A physicochemical investigation by Harwood (11) revealed that the conductivity of the C.E. at infinite dilution was of the same order as calcium sulphate, and concluded that the colloidal ion of the C.E. must possess a mobility similar to that of a sulphate ion: no conclusions could be drawn from his results as to the basicity of the acid complex. A further publication by Haas and Russell-Wells (12) described attempts to remove the sulphate residues without breaking down the carbohydrate complex, these/

these were unsuccessful but various fractions were obtained, which were separated by dialysis. Fructose was deemed present on the basis of the Seliwanoff test; but it may be pointed out that both this and the Bredereck test are not specific for fructose, since anhydro sugars such as 3:6-anhydro-l-galactose give positive results (13). Treatment with hydrochloric acid until ketose-colour reactions were no longer obtained followed by osazone formation resulted in the isolation of glucosazone, indicating the presence of some glucose in the products of hydrolysis. Potassium hydrogen saccharate was also isolated from the potassium salt of the H.E. after mucic acid formed by oxidation with nitric acid had been removed. It was shown that complete removal of the sulphate residues with alkali could not be achieved, only 20% removal being recorded in 16 hours with 3% sodium hydroxide at 110°.

A slightly different method of extracting the polysaccharides of Irish moss was employed by a Canadian investigator, M.R.Butler (14) and results which differ somewhat from those of Haas and his school are reported by her. Her standard extract contained 18.6% of ash and the potassium content was relatively high, up to 12%; calcium being about 3%. It should be pointed out here that differences may be due/

due to the fact that her material was collected in Eastern Canada. Butler found 26.8% for the sulphate after hydrolysis and 10.9% in the ash, the ratio being much greater than 2:1 demanded by Haas' theory. By dialysing against appropriate salts Butler prepared pure potassium, calcium and ammonium salts of the carbohydrate ethereal sulphate, and in the first two the 2:1 sulphate ratio held good. She made the suggestion that the constitution of the polysaccharide was represented by:

$$[R(O.SO_2.OK)_2]_n \quad \text{or} \quad [R(O.SO_2.OK)_3]_n$$

Since the sulphate content of the pure potassium salt was 28% a formula

$$[R.O.SO_2.OK]_n$$

would give a 'molecular weight' for R of *ca* 200. This would be in fair agreement with R as a monosaccharide residue. Nelson and Cretcher (15) have pointed out that another polysaccharide ethereal sulphate isolated from Macrocystis pyrifera can be represented as a chain of hexose units each carrying one sulphate residue.

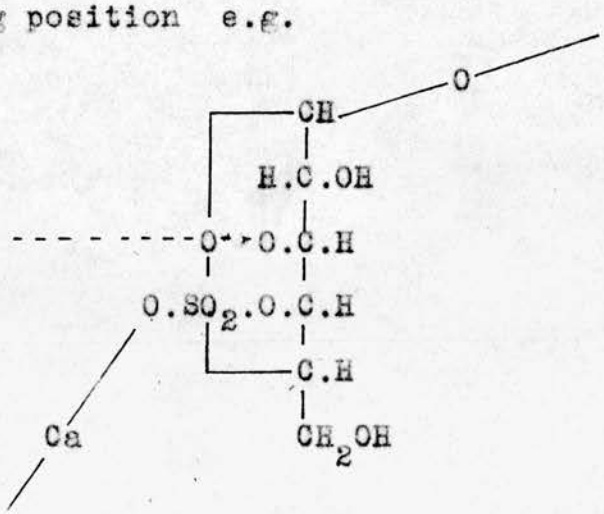
Dillon and O'Colla (16) have reported the results of experiments on the acetolysis of carragheen extract with acetic anhydride and sulphuryl chloride These workers obtained two sulphate-free acetates composed exclusively of galactose units (galactans).

Buchanan/

Buchanan and E.G.V.Percival (17) have attempted to throw further light on the Chondrus Crispus polysaccharides by the use of the methylation method. The hot extract H.E. was selected for this purpose and their results may be summarised here since they have a bearing on some of the work described in this thesis on a cold extract. The results of ash determinations (18.6%) and analyses, and estimations of the sulphate content of the ash (12.5%) and of the H.E. (23.8%) were in good agreement with those reported by Haas. The proportion of galactose in the H.E. was estimated as 37% but apart from the isolation of glucosazone, no evidence as to the composition of the non-galactosic portion of the hydrolysis products was obtained. It is to be noted that the Bredereck test (18) was negative, indicating the probable absence of fructose, so that the presence of glucose was considered likely, in agreement with Haas' findings. The proportions of pentose and methyl pentose were very small (2.4% and 1.2% respectively in the galactose free syrup).

Acetylation of the H.E. was impracticable by the usual methods. Methylation was tedious owing to the necessity of dialysis after each process, but a product was eventually obtained containing OMe, 14.2%. The product still contained ash (17.7%) and sulphate (27%); the ash still containing a high proportion/

proportion of calcium (20%). Hydrolysis and acetylation of this product gave on fractional distillation, a monomethyl and a dimethyl hexose acetate, from both of which on complete methylation tetramethyl galactopyranose anilide could be prepared. The acetates gave, on suitable treatment, galactosazone and 6-methyl galactosazone indicating the original acetates to be 2-methyl galactose tetraacetate and 2:6-dimethyl galactose triacetate. From these facts it is suggested that, as far as the galactosic portion of the molecule is concerned, the positions 2 and 6 are free, that the units are probably pyranose in character since the polysaccharide is fairly stable, so that these galactopyranose units are linked by position 1 (since the polysaccharide is non-reducing) and either position 3 or 4, the ethereal sulphate group being carried on the remaining position e.g.



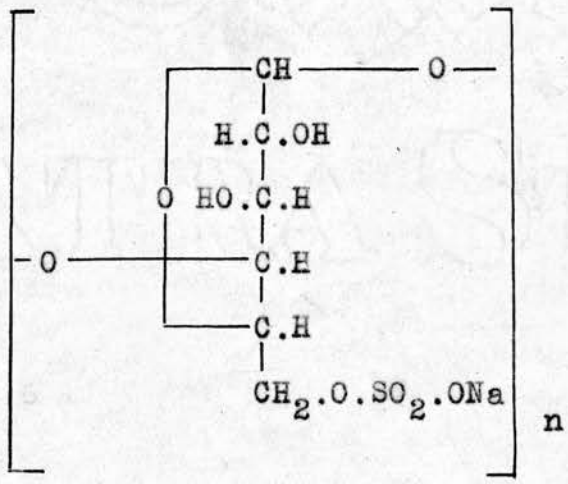
The full methoxyl content for a dimethyl galactose ethereal/

ethereal sulphate is never reached and it would seem that it is the primary alcohol group in position 6 which is sometimes deficient in methoxyl since 2-methyl as well as 2:6-dimethyl galactose was isolated; it was considered probable that the sulphate residue had a shielding effect preventing in some cases the methylation of this group.

Finally one may mention the results which Hassid (19) has obtained with another algal polysaccharide ethereal sulphate obtained from Irideaae laminarioides, since this is an example where the experimental difficulties appear to be less pronounced than in the ethereal sulphate under discussion. The polysaccharide present as a sodium salt gave an ash containing 5.8%S, whereas the total S was 11.5%. The sulphate was not ionised; hydrolysis gave galactose exclusively (54%). Acetylation yielded a diacetate; Hassid reported that the ethereal sulphate grouping could be removed, with 0.5N-sulphuric acid or with 5% baryta, without destroying the carbohydrate complex. Methylation of the galactan sodium sulphuric ester gave a dimethyl ether still carrying the sulphate residue from which a crystalline dimethyl methylgalactoside was obtained on hydrolysis; the constitution of this product was however not determined. The sulphate-free galactan was caused to yield a trimethyl

galactan/

galactan from which a trimethyl galactose was obtained on hydrolysis. This yielded no osazone so that the hydroxyl group on C_2 was presumed to be replaced by methoxyl. On the assumption of a pyranose structure and the production of a dimethoxy methylglutarate on oxidation it is concluded that one methoxyl group occupies position 6. Hassid rejects a 1:3-linkage of the galactose units and his idea of the constitution of the sodium sulphuric ester of this galactan is given by



where n = 6

There is an obvious weakness in the assumption that the linkage may not be at C_3 since this is common in galactose containing polysaccharides e.g. agar (20), damson gum (21) and gum arabic (22), and the molecular size seems very small. The most striking features of this polysaccharide in comparison with the carrageen polysaccharides are the ease of removal of the ethereal sulphate residue by acid; the surprising fact that although alkali removes the ethereal sulphate methylation with sodium hydroxide and methyl sulphate, in /

which process slight alkalinity is difficult to avoid, does not; and the ease of acetylation. This last point would certainly be in harmony with Hassid's conclusions of the relatively small molecular size.

Discussion of Results

The non-reducing polysaccharide obtained from carrageen after soaking for 24 hours in cold water following a preliminary soaking for one hour had $[\alpha]_D^{18°}$ +50°, ash, after prolonged dialysis, 22.4%. Analysis of the ash showed Ca, 5.5; Na, 13.7; K, 24.5; SO_4, 63.8%, whereas the amount of SO_4 obtained on hydrolysis was 35.1% . A trace of ammonia was evolved from C.E. on boiling with sodium hydroxide solution. The C.E. was hydrolysed with oxalic acid and 43% of galactose estimated as phenylmethylhydrazone was found in the hydrolysed mixture of sugars, corresponding to 34% in the polysaccharide. The polysaccharide was found to contain only ca 1% of pentosan. The corresponding figures for H.E. are $[\alpha]_D^{18°}$ +63°, ash, 18.7% containing Ca, 29.9; Na, 1.0; K, 2.5; SO_4, 66.6%; total sulphate 23.8%. The galactose content was estimated to be 36.9%.

It seems likely that the physical differences between the two polysaccharides are in all probability due to the difference in calcium content. It may be suggested that, with such a bivalent ion linking chains of sugar ethereal sulphates together, the tendency to form a gel structure would be more pronounced/

nounced in concentrated solution, and this may explain the differences in solubility. In any event H.E. is certainly soluble in cold water to some extent (at least 0.3%).

This view of the essential identity of C.E. and H.E., apart from the mineral constituents, was confirmed by the fact that treatment of a gel of H.E. with sodium oxalate gave a solution of low viscosity. Conversely dialysis of C.E. with calcium chloride gave a product similar to H.E., ash 21% containing Ca, 30.7%.

An investigation of the sugars recovered from the phenylmethylhydrazone mixture after removing the galactosephenylmethylhydrazone failed to reveal any new facts. Glucose was not present in sufficient quantity to yield the readily crystallisable β-methylglucoside tetraacetate or tetramethyl glucopyranose. This method was indeed applied to a similar "galactose free syrup" obtained from the H.E., i.e. acetylation, treatment with hydrogen bromide in acetic acid followed by methyl alcohol and silver carbonate to yield crystalline β-methylglucoside tetraacetate, ~~from which crystalline β-methylglucoside was obtained on deacetylation~~. This represents the first true glucose derivative isolated from

a/

a carrageen polysaccharide. A small amount of tetramethyl glucopyranose was also isolated on appropriate treatment of the mother liquors.

Very mild treatment of the C.E. with 0.013 N-sulphuric acid resulted in the isolation of a faintly reducing white powder, $[\alpha]_D^{18°}$ +66° in water, ash, 22.9%. Hydrolysis yielded 52% of galactose (as phenylmethyl-hydrazone).

Since the proportion of galactose estimated in the polysaccharide is based on the acid hydrolysis of an ethereal sulphate, it might not be considered impossible that sugar ethereal sulphates undergo changes in composition during acid hydrolysis: if for example anhydrosugars were formed the figure for galactose would be incorrect. The "galactose-free syrup", ignorance of the structure of which has been stressed, might also result in this way, especially as it gives ketose colour tests, which Forbes and Percival (13) showed to be the case with 3:6-anhydrogalactose. Experiments were carried out therefore on the course of hydrolysis of β-methyl-galactoside sulphate with acid and the rate of hydrolysis and the rate of fall of the specific rotation agreed with observations made on α-methyl-galactoside under the same conditions. If 3:6-anhydro-β-methylgalactoside were initially produced the/

the rotation would fall since this substance has $[\alpha]_D$ -110° and if 3:6-anhydrogalactose were formed the final rotation should be +24°. Furthermore galactosazone was isolated in good yield and no trace of the readily crystallised 3:6-anhydrogalactosazone was found.

As in the case of H.E. acetylation was unsuccessful and methylation was resorted to. The highest methoxyl content recorded was OMe, 14.5% and the methylated polysaccharide had $[\alpha]_D^{18°}$ +22° in water, ash 17.1%, SO_4 (by hydrolysis) 24.2%.

Hydrolysis with oxalic acid was followed by acetylation and the syrupy mixture of partly methylated galactose acetates (OMe, 14.7%) on complete methylation and treatment with aniline readily gave the characteristic tetramethyl galactopyranose anilide.. Since both 2-methyl β-methylgalactoside and 2:6-dimethyl methylgalactoside have been described as crystalline by Oldham and Bell (23) an attempt was made to isolate these derivatives. The usual technique with hydrogen bromide in acetic acid followed by methyl alcohol in the presence of silver carbonate yielded a mixture of acetylated, methylated methylgalactosides (OMe, 23%, $[\alpha]_D^{16°}$ +6°) which were deacetylated with dimethylamine. A crystalline/

crystalline compound was thus isolated, m.p. 130°, $[\alpha]_D^{15°}$ +1.5° in water which analysis showed to be a monomethyl methylgalactoside. These constants are in good agreement with the values recorded by Oldham and Bell for 2-methyl β-methylgalactoside, namely m.p. 131-2°, $[\alpha]_D^{17°}$ +1.7°. Since no 2-methyl β-methylgalactoside was available for direct comparison and the properties of 6-methyl β-methylgalactoside have never been described and it was thought likely that this compound might well be present having regard to the isolation of 2:6-dimethyl galactose from the H.E., 6-methyl β-methylgalactoside was prepared from the 6-methyl galactose of Freudenberg and Smeykal (24). The compound had m.p. 114-115°, $[\alpha]_D^{13°} \pm 0°$;, obviously not the one isolated from C.E.

Experiments on the C.E. having indicated the possibility of obtaining a degraded polysaccharide containing a slightly higher proportion of galactose than the original polysaccharide, work on similar lines was instituted on the methylated C.E.

Hydrolysis with 0.013N-sulphuric acid followed by neutralisation with barium carbonate and precipitation with alcohol gave two fractions, one insoluble (A) and a soluble slightly reducing friable solid (B). The properties and yields of this latter fraction were/

were variable owing to the difficulty of standardising the technique of precipitation.

Examination of A.- This solid, $[\alpha]_D$ +39°, had OMe, 13.3%, ash 16.8%. Like the C.E. itself further methylation of (A) failed to increase the methoxyl content. Hydrolysis with oxalic acid followed by glycoside formation and distillation in a high vacuum yielded two fractions:

(1) b.p. 160-180°(bath temp.)/0.05 mm., $n_D^{16°}$ 1.4700, $[\alpha]_D^{18°}$ +73° in water, OMe, 43%. Calc. for dimethyl methylgalactoside, $C_9H_{18}O_6$, OMe, 42%.

(2) b.p. 180-220°(bath temp.)/0.05 mm., $n_D^{15°}$ 1.4807, $[\alpha]_D^{15°}$ +79° in water, OMe, 29.5%. Calc. for a monomethyl methylgalactoside, $C_8H_{16}O_6$, OMe, 30%.

Both fractions on methylation, hydrolysis and anilide formation gave tetramethyl d-galactopyranose anilide in good yield.

Fraction (1) on hydrolysis, acetylation and the usual treatment with hydrogen bromide in acetic acid followed by the action of methyl alcohol in the presence of silver carbonate and then dimethylamine gave crystalline 2-methyl β-methylgalactoside, m.p. 130°.

In another experiment hydrolysis of (A) was followed by acetylation and distillation. After refractionation the following fractions were obtained:

(1)/

	Bath temp.	$n_D^{15°}$	% of distillate	%OMe
(1)	b.p. 154-165°/0.05 mm.	1.4760°	3.9	
(2)	165-176° "	1.4580	69.0	13.0
(3)	175-200° "	1.4601	14.0	7.9
(4)	Residue		10.0	

Repeated attempts were made to separate fraction (2) into a dimethyl and a monomethyl hexose acetate, but the product from three fractional distillations was still a mixture and showed b.p. 180-190° (bath temp.) /0.1 mm., $n_D^{18°}$ 1.4580°, OMe, 15.4%. Calc. for a dimethyl galactose triacetate, $C_{14}H_{22}O_9$, OMe, 18.6%. A crystalline monomethyl galactosazone was however obtained, m.p. 201-4°, not depressed on admixture with authentic 6-methyl galactosazone. It was not found possible to isolate a crystalline phenylhydrazone, although such a compound is readily obtained from 6-methyl galactose. It is therefore reasonable to claim that the 6-methyl galactosazone arose from 2:6-dimethyl galactose.

A portion of the sugars obtained by the deacetylation of this fraction was oxidised; difficulties were experienced in isolating the appropriate lactone owing to the presence of inorganic salts from the deacetylation process, but the isolation of a small yield of lactone of negative rotation $[\alpha]_D$ -25° may be taken as confirmation of the absence of a methoxyl residue on C_4, since

that/

that would inhibit the formation of a γ-d-galactonolactone of negative rotation.

Fraction (3) was examined next. After deacetylation and oxidation the sugar was converted into a lactone which had the characteristic properties of a d-γ-galactonolactone, hydrolysis occurring slowly and titration proceeding in the manner characteristic of such a case. Although, owing to the fact that purification by distillation of a monomethyl galactonolactone cannot be achieved because of the high boiling point, the negative rotation shows clearly that a γ-galactonolactone was present: $[\alpha]_D^{15°}$ -17° (initial, in water; c, 2.0); -15° (4 days, constant). From this impure lactone an amide was obtained via the corresponding ester which gave a negative Weerman test, indicating that the hydroxyl group on C_2 was occupied by a methoxyl residue.

Evidence is thus presented that the solid (A) on hydrolysis yields 2:6-dimethyl and 2-methyl galactose.

Examination of (B).- Fraction (B) had $[\alpha]_D^{18°}$ +14° and OMe, 18.6%. Hydrolysis yielded a syrup $[\alpha]_D^{17°}$ +37°, OMe, 19.6%, which was acetylated and distilled in a high vacuum as before. By this means the greater

portion/

portion distilled at 160-180°/0.01 mm. and had $[\alpha]_D$ +19°, OMe, 16.6%, and since complete methylation followed by hydrolysis and anilide formation gave tetramethyl galactopyranose anilide it would appear to be chiefly a dimethyl galactose triacetate. The dimethyl sugar obtained on deacetylation had $[\alpha]_D^{15°}$ +43° in methyl alcohol and this value, became -14° in 4 days in the presence of 1% hydrogen chloride. This reversal of sign is characteristic for d-galactose or any galactose derivative of which the hydroxyl group on C_4 is free so that it appears probable that since osazone formation gave an osazone containing but 7-8% of OMe that it is necessary to differentiate between 2:6- and 2:3-dimethyl galactoses, 2:5-dimethyl galactose being excluded on account of the formation of 2:3:4:6-tetramethyl galactose on methylation. Unfortunately in this case the highest m.p. obtained for the monomethyl galactosazone was 184-190°. 3-Methyl galactosazone is reported by Robertson and Lamb (25) to have m.p. 176-179°, so this possibility cannot be entirely set aside although no evidence for the presence of 2:3-dimethyl galactose was afforded by oxidation with nitric acid, esterification and amide formation, which should have yielded the readily obtainable d-dimethoxysuccinamide.

dimethoxysuccinamide.

It thus appears that (B) is more highly methylated than (A) and yields on hydrolysis chiefly a dimethyl galactose, probably 2:6-dimethyl galactose, it must be borne in mind that the amount of (B) obtained on the hydrolysis is only 1/3rd. to 1/4th that of (A).

To sum up the results of all the experiments with the methylated C.E., it seems that in the galactose portion, methylation appears to have confined itself to the hydroxyl groups on C_2 and C_6, no evidence of substitution on any other hydroxyl group having been proved. The proof that substitution does occur on C_2 is somewhat more rigid than that advanced for the H.E. (17), but the same general conclusions must be drawn, namely that the ethereal sulphate residue or the "polysaccharide link" may be on C_3 or C_4 if we assume pyranose galactose units. This may be taken as a working hypothesis. Although we do not know the effect of the ethereal sulphate residue on the stability of the polysaccharide one would not expect any galactofuranose units to be present.

The Stability of the Sulphate Residue in C.E.

One of the most noteworthy facts about the polysaccharide under investigation is the resistance of the sulphate group to hydrolysis by alkali. Haas (12) showed that in 3% sodium hydroxide solution at 110° hydrolysis proceeded to the extent of 10% in 1 hour, 20% in 16 hours and was estimated as complete in 54 hours under these conditions. Experiments were carried out in 4% sodium hydroxide at 100° and by estimating the liberated sulphate gravimetrically the results in the accompanying table were obtained.

Time in Hours	1	4.7	8.16	13	23	31.75	51.5
% Hydrolysis	14.5	40.2	50.3	57.4	79.3	90	98

These observations recalled to mind two other cases where alkaline hydrolysis was found to be difficult although the velocities of hydrolysis were not recorded. The first is to be found in a publication by the present author in 1938 (26) in which it is shown that 3-p-toluenesulphonyl 2:4:6-trimethyl α-methylgalactoside is difficult to hydrolyse with sodium methoxide, and the second concerns a more directly comparable case, namely, barium diacetone galactose-6-sulphate which was shown by E.G.V.Percival and Soutar (27) to resist hydrolysis by 8% sodium hydroxide during 6 hours at 100°, In the same paper it/

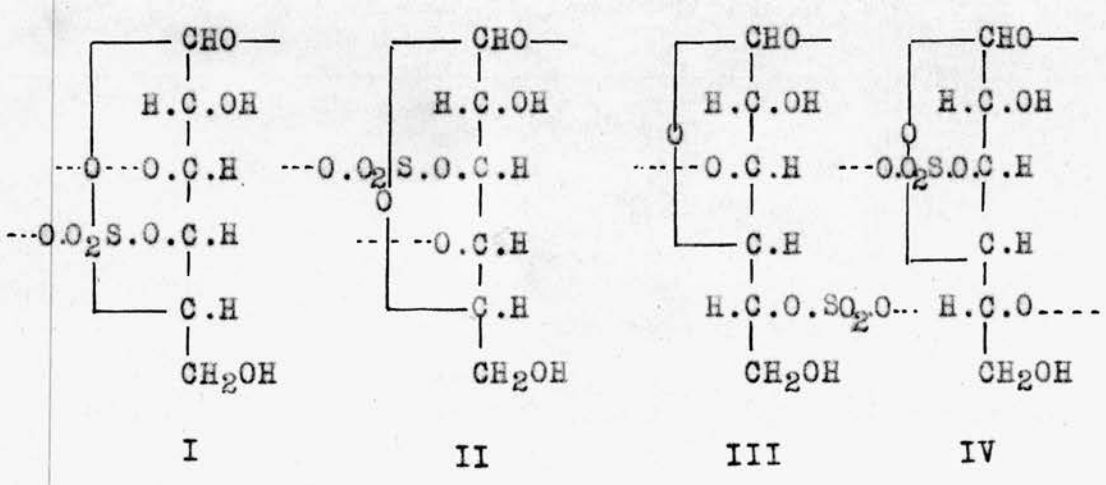
it is pointed out that barium glucose and barium galactose sulphates were hydrolysed completely in 5 minutes with 0.1 N-sodium hydroxide and that methylglucoside and methylgalactoside sulphates were also hydrolysed with alkali.

In order to secure a direct comparison between C.E. and a sulphate of simpler constitution potassium β-methylgalactoside sulphate was selected; Duff and E.G.V.Percival (28) having shown that alkaline hydrolysis of the barium salts of α- and β-methylglucoside sulphates and α- and β-methylgalactoside sulphates and α-methylmannopyranoside sulphate yields the corresponding 3:6-anhydromethylhexosides. Hydrolysis with 4% sodium hydroxide solution at 100° proceeded much more rapidly than for C.E. and was certainly complete in 2 hours, and the reaction is thus about 30 times as rapid in this case.

It will be observed that in the examples where hydrolysis with alkali is difficult, the removal of the sulphate or p-toluenesulphonate group is a "straight" hydrolysis since all the hydroxyl groups are substituted, whereas in the example just quoted an anhydromethylhexoside is produced and this appears to facilitate the fission of the sulphate group. One is thus enabled to make the suggestion that in the C.E. the sulphuric ester residue is not placed in a position in relation to an hydroxyl group so that a 3:6-anhydrogalactose/

galactose ring can be formed since in such a case alkaline hydrolysis would be much more rapid than is observed. A possible objection might be raised when it is recalled that when the galactan sodium sulphuric ester isolated by Hassid (19) was hydrolysed by alkali with the production of a galactan, no mention was made of the production of anhydro-rings. From the formula given by Hassid which carries a sulphate residue on C_6 and in which C_3 carries a free hydroxyl group it would be expected that a 3:6-anhydrogalactose would appear in the hydrolysis products. This is not the case, so the suggestion may be made that the hydroxyl group on C_3 is not free but is involved in a "polysaccharide link". It will be recalled that Hassid's only evidence for 1:4 linking was that this is common in other polysaccharides.

If we accept the evidence from the methylation experiments on Chondrus Crispus C.E. that the hydroxyl groups on C_2 and C_6 are free in the polysaccharide sulphate, the possibilities may be set out below:



It would be expected that (II) would hydrolyse to yield a 3:6-anhydrogalactose just as readily as if the sulphate residue were on C_6 and this formulation should therefore be discounted. (I) could however only form a 2:4-anhydro-ring or a 4:6-anhydride. Such rings have never been observed either with sulphates or p-toluenesulphonates, the latter giving as is well known when a free hydroxyl group is on an adjacent carbon atom ethylene oxide rings with or without Walden inversion. According to our present knowledge therefore such a sulphate as (I) would be resistant to alkaline hydrolysis and accordingly this is suggested as the most likely method of linking the galactose residues in C.E.

By way of confirmation that no new anhydro-rings are introduced on alkaline hydrolysis, a quantity of C.E. was hydrolysed with sodium hydroxide and the resultant product was found to yield 49.4% galactose on hydrolysis, which is a slightly higher proportion than that for the syrup obtained by the direct hydrolysis of the polysaccharide (43%). This would be expected if the splitting off of the sulphate residue by alkali only yields galactose and not an anhydrogalactose.

Both (III) and (IV) are considered improbable on account of their furanoside structures, and because one would expect them to yield anhydrides with ease/

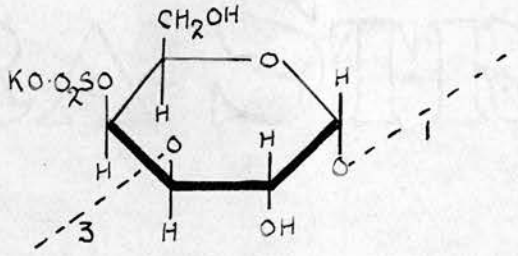
ease. (III) could yield a 5:6-oxide ring and (IV) a 2:3-oxide ring, and although such ethylene oxide rings have not yet been obtained by the alkaline hydrolysis of sulphates as distinct from p-toluenesulphonates, there is no reason to doubt that they could be formed readily. (IV) might also yield a 3:6-anhydride, although Haworth and Smith (29) have shown that 3:6-anhydro-α-methylgalactopyranoside cannot be converted by a trace of acid into the corresponding furanoside as can the corresponding glucose derivatives, for steric reasons.

By way of criticism the suggestion might be made that during the methylation processes sulphate residues are replaced by methoxyl, and that the sulphate group might therefore have been located on C_2 or C_6. Against this it must be pointed out that in such a case the methoxyl content would tend to be higher than that for a polymerised anhydro dimethyl galactose, whereas in fact the theoretical value for such a compound is never reached.

Until evidence can be secured as to the structure of the sulphate-free polysaccharide, the progress of the study of which has had to be temporarily abandoned, it seems reasonable to postulate that the C.E. polysaccharide contains its galactose units mutually joined by/

by positions 1 and 3, in which it resembles other galactose-containing polysaccharides (20, 21, 22), and carrying the sulphuric ester group on C_4. This takes no account of the mode of union of the other unidentified sugars which are present.

The C.E. and the methylated C.E. both have positive rotations, so that in conjunction with the preponderance of d-galactose units the predominating linkage appears to be α- and the following is therefore suggested as the main, although not the only, building stone in the polysaccharide.



E_X_P_E_R_I_M_E_N_T_A_L

Preparation of Carrageen Extracts

A modification of the method described by Haas (9) was used and in this case three distinct extracts were obtained. The weed (300 g.) purchased in bulk from a well-known firm, was twice washed with cold water, drained, and steeped in water (5 litres) for 1 hour. The extract was filtered through muslin and the filtrate concentrated at 50°/20 mm. to 300 c.c. This was added drop by drop to ethyl alcohol (1 litre) with mechanical stirring to yield a fibrous, greyish white product, which was dehydrated with fresh alcohol, filtered and dried in a vacuum over calcium chloride. This was the Preliminary Cold Extract (25 g.). The weed was again washed twice with water and allowed to stand in water (5 litres) for 24 hours. The extract was similarly treated to yield 'Cold Extract' (C.E., 40 g.). The weed was washed in running water for 7 days and finally extracted with water (10 litres) in muslin bags on the steam-bath for 6 hours. The aqueous solution was removed and the process twice repeated and the combined extracts were treated in the same as the C.E. to give the 'Hot Extract' (H.E., 90 g.).

Properties of C.E. The C.E. was non-reducing to Fehling's solution. It was soluble in water and gave $[\alpha]_D^{18°}$ +50° in water (c, 0.5). 0.312 G., after prolonged dialysis, was incinerated to yield 0.0669 g. ash as sulphate = 22.4%.

Analysis of the Ash.- A quantitative analysis for sulphate, calcium, potassium and sodium was made. The ash (0.4140 g.) was dissolved in 2N-hydrochloric acid. The solution was filtered free from a very small amount of insoluble residue and made up to 100 c.c. with distilled water. This solution was used for the following determinations. The determination of sulphate (on 40 c.c. of the solution) was made gravimetrically by precipitation as barium sulphate (30). Calcium was estimated volumetrically (on 10 c.c.) by precipitation as calcium oxalate, filtration, and after redissolving the precipitate in dilute sulphuric acid the solution was titrated with standard potassium permanganate (31). This was repeated to give an identical result. The potassium (on 20 c.c.) after neutralisation was precipitated with sodium cobaltinitrite (32), and the sodium (on 10 c.c.) was estimated as sodium zinc uranyl acetate (33). The results are tabulated/

lated below:

	Ash %	C.E. % (Calc. from ash)
Sulphate	63.8	12.1
Calcium	5.5	1.2
Potassium	24.5	5.5
Sodium	13.7	3.1

Determination of Sulphate on C.E. after hydrolysis.* Dried C.E. (0.4447 g.) was treated with concentrated hydrochloric acid (10 c.c.) for 4 hours on a water-bath at 64°. The product was diluted and estimated for sulphate gravimetrically by precipitation as barium sulphate (0.3794 g.) (30) (SO_4, 35.1%).

Comparison of H.E. and C.E.- Addition of potassium oxalate to a solution of H.E. gave a precipitate of calcium oxalate and a much less viscous solution.

C.E. (2 g.) in water (50 c.c.) was dialysed against a saturated solution of calcium chloride for 10 days using a cellophane membrane. The resulting product was then dialysed against running water for 5 days. The product on evaporation to dryness gave ash 20.7%; Ca, 30.7% and resembled the horny appearance of H.E. when isolated in this way. Furthermore on treating 0.1 g. with water (2 c.c.) the substance slowly gave a viscous solution, although complete solution was not achieved. A control experiment with H.E. and C.E. confirmed this in a qualitative manner.

*C.E. (3 g.) was boiled with 2N-sodium hydroxide and a trace of ammonia was evolved

Pentose Estimation.- The pentose content was estimated according to the method described by Meyer (34). The amount of pentose was calculated from the weight of furfural phloroglucide obtained using the appropriate factors. C.E. (4.0182 g.) gave 0.0368 g. of furfural phloroglucide which corresponds to 0.02 g. furfural indicating the presence of 1% pentose.

Partial Hydrolysis of C.E.

The extract (3.5 g.), $[\alpha]_D^{18°}$ +50° was hydrolysed with 0.013N-sulphuric acid (150 c.c.) at 100° for 3.5 hours, cooled and filtered. The filtrate was neutralised with barium carbonate, filtered, the precipitate extracted with water and the filtrate and extracts evaporated to yield a light brown tacky solid which was very reducing. It was purified by solution in hot water and precipitation with alcohol. Filtration and drying gave a slightly reducing white powder. Further additions of alcohol to the filtrate gave additional yields - the final product being a yellowish syrupy solid. Yield of white powder 3 g., ash, 22.9%, $[\alpha]_D^{18°}$ +66° in water (c, 0.7)

Complete Hydrolysis of above Product with N/2-Oxalic Acid.- A portion of the above white powder (1.18 g.) was thoroughly dried (1.0 g.) and refluxed with N/2-oxalic/

oxalic acid (30 c.c.) for 26 hours. An inorganic precipitate (0.03 g.) was deposited and removed; the filtrate was cooled and neutralised with calcium carbonate, warmed and filtered. The precipitate was extracted several times with water. The filtrate and extractions on evaporation at 50°/16 mm. gave a syrup admixed with solid. Galactose was shown to be present and the amount was determined as galactosephenylmethylhydrazone. The syrupy mixture, dissolved in water (7 c.c.), was treated with ethyl alcohol (7 c.c.), acetic acid (0.1 c.c.) and phenylmethylhydrazine (0.75 c.c.). The solution was allowed to stand for 3 days at -3°. Crystalline galactosephenylmethylhydrazone was deposited and removed by filtration. A further yield was obtained from the filtrate on standing for 2 days. Total yield after drying over phosphoric oxide in a vacuum (0.79 g.). [Comparative preparations made from galactose showed that 1.006 g. galactose gave 1.55 g. galactosephenylmethylhydrazone (Theory 1.58 g.)]. Several of these estimations were carried out on C.E. with similar results. Calculated amount of free galactose in the hydrolysis products of the dried white powder = 52%.

Typical Preparation of the "Galactose free Syrup"

After washing the galactose phenylmethylhydrazone thoroughly with water the remaining sugars in the filtrate/

trate and washings were recovered according to the method of Lüdtke (35). The combined filtrate and washings were evaporated to 20 c.c. and treated with ethyl alcohol (15 c.c.) and benzaldehyde (2 c.c.) under reflux for 4 hours. The solution was cooled in ice for 15 hours, filtered and the precipitate thoroughly washed with water. The filtrate and washings were evaporated to 20 c.c., thoroughly washed with ether and evaporated at 50°/16 mm. to yield a viscous syrup.

Osazone Formation.- The syrup was dissolved in water (2 c.c.) and warmed with phenylhydrazine (0.3 c.c.), acetic acid (0.3 c.c.), a little sodium acetate and a trace of sodium bisulphite. An amorphous osazone was deposited. All attempts at separation and crystallisation failed.

Complete Hydrolysis of C.E.

C.E. was completely hydrolysed with sulphuric and oxalic acids and in each case the galactose was removed as the phenylmethylhydrazone as described on page 91.

1. With N/2-Sulphuric Acid.- C.E. (2 g.) was treated with N/2-sulphuric acid (160 c.c.) at 100° for 5 hours to yield a light brown very reducing solid which gave very strong tests for ketose. Conversion to galactosephenylmethylhydrazone gave 1.03 g./

1.03 g. (m.p. 184°) which is equivalent to 34% of galactose in the dried C.E.

Treatment of the filtrate as in the previous case (page 91-92) gave a mobile colourless syrup (1.3 g.) which gave faint tests for a ketose.

Several attempts were made to prepare a pure osazone, but in every case mixtures were obtained.

2. With N/2-Oxalic Acid.- C.E. (Moisture content 11.8%, 2.46 g. after drying) was refluxed with N/2-oxalic acid (72 c.c.) for 20 hours. 0.05 G. of inorganic solid (shown to be calcium sulphate) was deposited. Yield of galactosephenylmethylhydrazone 1.28 g. which corresponds to 34% of galactose in the dried C.E. or 43% in the hydrolysed mixture of sugars.

Treatment of the filtrate gave a small quantity of syrup from which no identifiable osazone could be isolated.

Isolation of β-Methylglucoside Tetraacetate from H.E.

H.E. (15 g.) in water (400 c.c.) was hydrolysed for 24 hours at 100° with oxalic acid (16 g.). The product worked up in the usual way was treated with phenylmethylhydrazine (8 g.). The residue was treated with benzaldehyde (20 c.c.) in alcohol (50 c.c.) and after removing the benzaldehydephenylmethylhydrazone/

methylhydrazone by filtration and excess of benzaldehyde with ether a syrup (3 g.) was obtained on evaporation having $[\alpha]_D^{18°}$ +9° in water (c, 1.4).

By comparing in a colorimeter the colour developed with 4% ammonium molybdate solution acidified with acetic acid with that developed by fructose it was estimated that this syrup contained about 17% of a ketose.

The syrup (2.5 g.) was acetylated in the usual way, and the acetate converted into the acetobromo-compound with acetic acid-hydrogen bromide. Treatment with dry methanol and silver carbonate yielded a product from which crystals (0.16 g.) of β-methylglucoside tetraacetate were obtained, m.p. 104°, not depressed on admixture with an authentic specimen, $[\alpha]_D^{17°}$ -18.6° in chloroform (c, 0.7).

Isolation of Tetramethyl Glucose.- The residual syrup had $[\alpha]_D^{15°}$ +7° in chloroform, and was methylated first with dimethyl sulphate and sodium hydroxide, then by silver oxide and methyl iodide, $n_D^{13°}$ 1.4495, OMe, 60.2%, which on hydrolysis partially crystallised to give tetramethyl glucopyranose, (0.05 g.), m.p. 85°.

Attempted Isolation of β-Methylglucoside Tetraacetate from C.E.

Two attempts on a galactose-free syrup (2-3 g.) obtained from the cold extract gave neither β-methylglucoside/

glucoside tetraacetate nor tetramethyl glucopyranose. The galactose free syrup had $[\alpha]_D^{17°}$ +9° in water and colorimetric observations indicated the presence of 20-22% of a ketose.

The Course of Hydrolysis of β-Methylgalactoside Sulphate with Acid

Barium β-methylgalactoside sulphate (0.2517 g.) was dissolved in water, just sufficient 0.84 N- sulphuric acid was added to precipitate the barium and the filtered solution diluted with sufficient water to make the sulphuric acid (0.1 N) (17 c.c.), and the mixture heated at 95°. Samples were withdrawn at definite intervals and the rotation measured:

Time (minutes)	$\alpha_D^{16°}$	$[\alpha]_D^{16°}$
0	0°	0°
30	.02	+2
110	.04	4
210	.19	22
345	.32	37
595	.39	45
745	.46	54
905	.46	54
1005	.50	59
1405	.54	66

After neutralisation and treatment with phenylhydrazine acetate galactosazone, m.p.189°, was isolated (10.9%). No 3:6-anhydrogalactosazone could be detected.

The Course of Hydrolysis of α-Methyl galactoside with Acid

α-Methylgalactoside (0.3735 g.) was dissolved in 0.1N-sulphuric acid (20 c.c.) and the rotation measured a definite intervals.

Time (minutes)	$\alpha_D^{16°}$	$[\alpha]_D^{16°}$
0	+3.35°	+179°
85	3.22	172
185	2.99	160
320	2.76	145
570	2.44	130
720	2.26	121
880	2.19	117
980	2.06	110
1380	1.76	95
1425	1.76	95
1485	1.76	95

Attempted Acetylation of the C.E.

Crude C.E. (4.5 g., ash 22.4%) was dissolved in the minimum quantity of water (120 c.c.) and treated with pyridine (200 c.c.) and acetic anhydride (75 c.c.) under reflux at 80° for 5 hours. No precipitate was deposited on cooling and the solution was evaporated to dryness, taken up with a small quantity of water and the product precipitated with alcohol as a white powder (3.5 g., ash 18.3%).

Purification of a small portion, (0.8 g.) by solution in water (100 c.c.) and dialysis in a parchment bag against running water for 48 hours, followed by evaporation to 50 c.c. and precipitation with alcohol, gave a white powder (Found: $CH_3.CO$, 6%).

Attempted Deacetylation and Methylation

The crude acetate (2.5 g.) was dissolved in warm water (80 c.c.), dimethyl sulphate (80 c.c.) and 30%/

30% potassium hydroxide (260 c.c.) were added in one tenth portions at intervals of 10 minutes with mechanical stirring at 75°. The mixture was heated to 100° for a few minutes, cooled, neutralised with acetic acid and dialysed in parchment bags against a rapid stream of running water, until free from sulphate (3 days). Evaporation gave a transparent glass (1.5 g.) OMe, 7.3%.

Direct Methylation of the C.E.

A modification of the method used by Baldwin and Bell (36) in the methylation of galactogen, was found to give the best results. The crude C.E. (5 g. ash 22.4%) was dissolved in the minimum quantity of water (100 c.c.) and mechanically stirred at 50°. To this eight lots each of methyl sulphate (15 c.c.), and 30% potassium hydroxide (60 c.c.) were added every 2 minutes, followed by eight lots each of methyl sulphate (7.5 c.c.) and 30% potassium hydroxide (18.5 c.c.) every 10 minutes. The temperature was raised to 100° for a few minutes and the mixture cooled in ice, neutralised with acetic acid and dialysed as in the previous experiment (the time varying in different experiments from 28 hours - 3 days). The dialysed liquid was evaporated at 50°/16 mm. to yield a horny scaly solid admixed with some inorganic material (6 g.) OMe, 7.6%. This was dissolved in water (300 c.c.) and remethylated with ten lo each of methyl sulphate (8 c.c. and 30% potassium hydroxide (26 c.c.)/

(26 c.c.) every 10 minutes at 50°. After treatment as above a transparent solid (OMe, 9.9%) was obtained The methylation was repeated a third time with the addition of double the quantities of methylating agents to that used in the second case. Dialysis for 6 days was found to be necessary. A hygroscopic transparent glass (3 g.) was obtained (OMe, after thorough drying, 10.6%, Ash, 19.7%).

In subsequent methylations on C.E. by methylating two 5 g. lots and combining the methylated product for the second methylation, it was found possible to increase the yield to 9 g. (OMe, 10.7%).

Attempted Acetylation of the Methylated C.E.

The methylated C.E. (3 g., OMe, 10.6%) was dissolved in the minimum quantity of water (75 c.c.) and acetylated under the same conditions as the unmethylated C.E. (page 96). The filtrate was evaporated as before to give a white powder on drying. A second treatment of the alcohol gave a further yield. Total yield 3.2 g. (Ash, 17.3%). A small quantity was purified by dialysis to give a white powder (OMe, 14.5%, CH_3CO, nil).

Repeated Methylation

The crude product (OMe, 14.5%, 1.5 g.) was dissolved in warm water (50 c.c.) dimethyl sulphate (40 c.c.) and 30% potassium hydroxide (130 c.c.) were added in one tenth portions at intervals of 10 minutes/

minutes with mechanical stirring at 75°. The mixture was worked up as in the previous case to give a transparent glass (1.5 g., OMe, 14.5%) $[\alpha]_D^{18°}$ +22° in water (c, 0.8). Ash, 17.1% SO_4 in ash 51.6% SO_4 by hydrolysis 24.2%.

Hydrolysis and Acetylation

The above methylated C.E. (3 g., OMe, 14.5%) was completely hydrolysed with oxalic acid (N/2) and the product acetylated with pyridine (32 c.c.) and acetic anhydride (20 c.c.) at 100° for a few minutes. The mixture showed signs of darkening and was, therefore, cooled and allowed to stand at room temperature for 3 days. The mixture was poured into water (400 c.c.), the aqueous solution washed four times with chloroform, the chloroform solution washed three times with dilute sulphuric acid, once with water and finally dried over anhydrous sodium sulphate. Removal of the solvent gave a syrup which on distillation under a high vacuum yielded one fraction: bath temp. 175-220°/2.5 mm. 1.16 g. OMe, 14.7%.

Deacetylation

The syrup (0.3 g.) was dissolved in dry chloroform (4 c.c.) and allowed to stand for 3 hours at room temperature in contact with sodium methylate (4 c.c.; 0.2 g. Na dissolved in dry methyl alcohol 25 c.c.).

25 c.c.). The mixture was then shaken with water (12 c.c.) containing acetic acid (0.2 c.c.) several times and the aqueous extracts washed once with chloroform and evaporated at 150°/16 mm. to yield a brown mobile syrup (0.17 g.).

Complete Methylation and Anilide Formation

The above syrup (0.15 g.) was subjected to two Purdie methylations. It was dissolved in methyl iodide (15 c.c.) and 5 additions of silver oxide (5 g. in all) were made at intervals of 1 hour, the solution being kept at 45°. The mixture was then filtered and the silver residues extracted 3 times under reflux with chloroform. Combined filtrate and extracts on evaporation yielded a pale yellow syrup. The glycosidic methoxyl group was removed by heating the syrup in 7% hydrochloric acid (8 c.c.) at 100° for 2 hours. The solution was neutralised with barium carbonate, alcohol (50 c.c.) was added to precipitate barium salts, which were filtered off and extracted with hot alcohol. The filtrate and extracts were evaporated at 50°/16 mm. The residue was extracted three times with ether and from the filtered extracts a pale yellow syrup was obtained on evaporation. The anilide was prepared from this syrup by heating with aniline (0.1 c.c.) in alcohol (8 c.c.) at 90° for 3 hours. Nucleation with a crystal/

crystal of tetramethyl galactose anilide gave crystalline tetramethyl galactose anilide, m.p. 195°. Mixed m.p. with an authentic specimen showed no depression.

Deacetylation and Formation of 2-Methyl β-Methylgalactoside

A further portion of the acetylated syrup (OMe, 14.7%; 0.5 g.) was dissolved in acetic acid (1 c.c.) and was treated with acetic acid saturated with hydrogen bromide (1.5 c.c.) for 3 hours. After the addition of more chloroform, washing with water and sodium bicarbonate solution, drying and removal of solvent, the syrupy acetobromo-compound was dissolved in dry methyl alcohol and shaken with silver carbonate for 12 hours. Removal of the solvent yielded a syrup (0.4 g.; OMe, 23.0%) $[\alpha]_D^{16°}$ +6° in chloroform (c, 0.7).

This syrup (0.3 g.) was dissolved in dry methyl alcohol (5 c.c.) and treated with dimethylamine in methyl alcohol (1 g. in 5 c.c.) and heated for 4 hours under pressure. The mixture was then taken to dryness, decolourised with charcoal, and gave a light syrup which was subjected to distillation under high vacuum in order to remove all the dimethyl acetamide. The resulting syrup crystallised on standing. Purification of the crystals by refluxing with ether, followed by recrystallisation from

ethyl /

acetate gave white needles of 2-methyl β-methylgalactoside, m.p. 130°, $[\alpha]_D^{15°}$ +1.5° in water (c, 1.0)
Analysis gave C, 45.9; H, 7.7; OMe, 28.9.
Calc. for 2-Methyl β-methylgalactoside, $C_8H_{16}O_6$:
C, 46.1; H, 7.75; OMe, 29.8%.

Preparation of 6-Methyl β-Methylgalactoside

6-Methyl galactose m.p. 125° (1 g.) was treated with pyridine (5 c.c.) and acetic anhydride (3 c.c.) at room temperature for 2 days. The product was isolated by pouring into water and extraction with chloroform, the solution was washed with dilute sulphuric acid to remove pyridine, followed by sodium bicarbonate solution. After drying and removal of the solvent the acetate (1.2 g.) dissolved in glacial acetic acid (2 c.c.) was allowed to stand for 3 hours with acetic acid saturated with hydrogen bromide at 0° (3 c.c.). At the end of that time chloroform (15 c.c.) was added and the mixture was poured on to ice, more chloroform was added and the solution was washed with sodium bicarbonate solution and dried over sodium sulphate. The syrup obtained after removal of the solvent at 35°/15 mm. was shaken for 12 hours with silver carbonate in anhydrous methanol. The product ultimately isolated in the usual way readily crystallised, and was recrystallised from a mixture of ethyl acetate and/

and light petroleum (b.p. 60-80°). M.p. 114-115°, $[\alpha]_D^{13°} \pm 0°$ in water (c, 0.7).

Found: C, 46.4; H, 7.8; OMe, 28.5.

$C_8H_{16}O_6$ requires

C, 46.1; H, 7.75; OMe, 29.8%.

Hydrolysis of the Methylated C.E. with 0.013N-Sulphuric Acid

The methylated C.E. (6 g., OMe, 14.5%) was dissolved in 0.013N-sulphuric acid (110 c.c.) and heated for 2.5-3 hours at 100°. A dark precipitate (0.11 g.) was deposited and removed. The cooled filtrate was neutralised with barium carbonate, filtered, and filtrate and washings were evaporated to 100 c.c. and then added drop by drop to alcohol (1 litre). A white precipitate was obtained, which was filtered, dehydrated in fresh alcohol, washed with ether and dried in vacuum. (5 g., OMe, 13.3%) $[\alpha]_D^{17°}$ +39° in water (c, 0.5). Ash, 16.8%. Henceforth this will be called Fraction (A).

The filtrate and washings were combined and evaporated at 50°/16 mm. to give a white solid (shown to be calcium sulphate) admixed with a reducing syrup. Extraction for some hours under reflux with alcohol and removal of the solvent gave/

gave a cream friable reducing solid (0.5 g.) (Fraction B) which turned syrupy on standing, $[\alpha]_D^{18°}$ +14° in water (c, 1.1).

Repetitions of this hydrolysis on different specimens of methylated C.E. with slightly different methoxyl content gave relatively different yields of Fraction (A) and (B):

Hydrolysis	C.E.	Yield (A)	OMe %	Yield (B)	OMe %	Propor.A/B
2	9 g.	4.1 g.	12.9	1.676g.	18.6	2.4/1
3	7 g.	2 g.	11.0	2 g.		1/1
4	6 g.	4.7 g.	13.2	1.2 g.		4/1

Attempted Methylation of Fraction (A)

Fraction (A) (2.5 g.) was dissolved in the minimum quantity of water (50 c.c.) and treated with methyl sulphate (5.5 c.c.) and 30% potassium hydroxide (15 c.c.) every 10 minutes 10 times at 50-70° in the usual way. The product was then dialysed for 4 days and worked up as in previous methylations to yield a mica-like glass (1.5 g.) (OMe, 13.4%).

Hydrolysis of Fraction (A) with N/2-Oxalic Acid

Fraction (A) (2.5 g.) was hydrolysed with N/2-oxalic acid (65 c.c.) for 20 hours at 90°. On cooling an inorganic precipitate was deposited (0.3 g.), this was removed by filtration and the filtrate and washings neutralised with calcium carbonate in the presence/

presence of charcoal, filtered, and evaporated at 50°/20 mm. to yield a syrup admixed with inorganic residue (0.6 g.). Thorough extraction with alcohol and removal of the solvent gave a syrup.

Conversion to the Glycoside

The syrup was treated with 5% methyl-alcoholic hydrogen chloride (60 c.c.) at 75° for 9 hours and the mixture neutralised with silver carbonate, filtered, precipitate extracted thoroughly, and the filtrate and extracts evaporated at 50°/16 mm. to yield a syrup (0.9 g.; OMe, 38.2%). Distillation at 0.05 mm. gave two pale yellow non-reducing syrups.

Fraction	Bath temp.	%OMe	$n_D^{16°}$	$[\alpha]_D^{15°}$ in water	Yield
1	160-180°	43	1.4700	+73° (c,1.1)	0.3327 g.
2	180-220°	29.5	1.4807	+79° (c,0.95)	0.2660 g.
				Residue	0.2700 g.
					0.8687 g.

Complete Methylation of Fraction (1)

A portion of the syrup (0.15 g.) was subjected to two Purdie methylations, as on page 100, to yield a pale yellow syrup. The glycosidic methoxyl residue was removed in the usual way (page 100) to yield a pale yellow mobile syrup. Anilide formation as on page 100 gave shining white needles (0.051 g.) m.p. 192-3°. A mixed m.p. with an authentic specimen of 2:3:4:6-tetramethyl galactose anilide showed no depression.

Hydrolysis, Acetylation, and Formation of 2-Methyl β-Methylgalactoside from Fraction (1)

The residue of fraction (1) was hydrolysed with N-sulphuric acid (5.5 c.c.) for 2.5 hours. The product was neutralised with barium carbonate, filtered, and the precipitate extracted several times. The filtrate and extracts on evaporation gave a clear syrup. This was acetylated, (page 99) poured into water and extracted with chloroform. It was then converted into the aceto-bromo-compound as on page 101. The resulting syrup was treated with dimethylamine for 40 hours. Dimethyl acetamide was removed by distillation in high vacuum. This gave rise to a syrup which crystallised on standing. Recrystallisation from ethyl acetate gave crystals, m.p. 130°, no depression on admixture with previous specimen.

Investigation of Fraction (2)

Fraction (2) (0.266 g.) was dissolved in N-sulphuric acid (10 c.c.) and the mixture refluxed until the rotation was constant, α_D +2.44° (45 mins.); ⟶ +2.24° (2 hours 25 mins.). Heating was discontinued and the mixture neutralised with barium carbonate, filtered and the precipitate extracted. The filtrate and extracts on evaporation at 50°/16 mm. gave a nearly colourless reducing syrup (OMe, 27.4%) which failed to crystallise. Glycoside formation, methylation, and anilide formation yielded tetramethyl galactopyranose anilide, m.p. 194°

Acetylation of Completely Hydrolysed Fraction (A)

Hydrolysed Fraction (A) (prepared as on page 104-105) (4.5 g.) was treated with pyridine (50 c.c.) and acetic anhydride (30 c.c.) and the product worked up in the usual way to yield a brown syrup (6.0 g.). Distillation in high vacuum gave 3 fractions:-

Fraction	Bath temp.	$n_D^{15°}$	Yield
1	154-165° /0.05 mm.	1.4760	0.23 g.
2	175-185°/0.02 "		2.88 g.
3	185-200° " "		2.33 g.
	Residue		0.52 g.
			5.96 g.

Fractions (2) and (3) were combined (5.2 g.) and subjected to further distillation in high vacuum.

Fraction	Bath temp.	$n_D^{15°}$	OMe	Yield
2b	165-176°/0.03 mm.	1.4580	13.0%	4.07 g.
3b	175-200°/ "	1.4601	7.9%	0.77 g.
			Residue	0.11 g.
				4.95 g.

Investigation of Fraction (2b)

An attempt was made to separate fraction (2b) (4.07 g.) by a further distillation in high vacuum

Fraction	Bath temp.	n_D	OMe	Yield
1c	150-160°/0.05 mm.	1.4595	13.9%	1.55 g.
2 c	160-171°/0.03 mm.	1.4582	12.7%	2.01 g.
			Residue	0.48 g.
				4.04 g.

Fraction (1c) (1.55 g.) was submitted to a further distillation with the following results

Fraction	Bath temp.	n_D	OMe	Yield
1d	130-135°/0.07 mm.			0.1 g.
2dd	150-160°/0.03 mm.	1.4559	13.4%	1.05 g.
3d	160°- "			0.2 g.
			Residue	0.14 g.
				1.49 g.

Reacetylation.- Since all attempts to obtain definite fractions by distillation were unsuccessful, fractions (2c), (2d), and (3d) (3.24 g.) were combined and acetylated as before to give a syrup (3.09 g.). Distillation gave the following results:

Fraction	Bath temp.	n_D	%OMe	$CH_3.CO$	
1e	180-190°/0.1 mm.	1.4580	15.4	45.2%	2.35g.
2e	195-230°/.03 "	1.4559			0.47
				Residue	0.26
					3.08g.

Investigation of Fraction (1e)

A portion of fraction (1e) (0.9739 g.) was deacetylated using Zemplen's method. The syrup was dissolved in dry chloroform (5 c.c.) and allowed to stand for 3 hours at room temperature in contact with sodium methylate (5 c.c.; 0.2 g. sodium dissolved in dry methyl alcohol 25 c.c.). The mixture was then

shaken with water (15 c.c.) containing glacial acetic acid (0.2 c.c.) several times and the aqueous extracts washed once with chloroform and evaporated at 150°/16 mm. to yield a mobile syrup (0.5 g.)

Osazone Formation.- A portion of the above syrup (0.16 g.) was converted into the osazone as on page 92. Heating for a short time caused the deposition of a small amount of oil. This was removed by decantation, and treatment with the minimum quantity of alcohol, followed by washing with ether gave a crystalline solid. Reheating of the phenylhydrazine mixture, followed by standing in the dark gave a number of precipitates of osazone which were filtered and washed with water containing a little acetic acid and then with water for several hours. After recrystallisatin these gave (0.04 g.), m.p. 196-8°. Mixed m.p. with authentic 6-methyl galactosazone (m.p. 195-6°) gave m.p. 196°.

This was repeated on a further portion of deacetylated syrup (0.78 g.) to give a purified osazone in the form of large crystals, m.p. 201-4°, which showed no depression on admixture with a more highly purified specimen of 6-methyl galactosazone (m.p. 206°)

Found: C, 60.0; H, 6.37; OMe, 8.6; N, 15.2.

6-Methyl galactosazone, $C_{19}H_{24}O_4N_4$ requires

C, 61.3; H, 6.45; OMe, 8.3; N, 15.0%.

Attempted Phenylhydrazone Formation.- Attempts to form a crystalline phenylhydrazone from a further portion of deacetylated Fraction (1e) were unsuccessful.

Lactone Formation.- Deacetylated Fraction (1e) (0.25 g.) was mixed with water (7 c.c.) and bromine (0.75 c.c.) added together with lead carbonate (3.5 g.) and the mixture allowed to stand 2 days; then kept at 35° for a short time when it was found to be non-reducing. It was neutralised with silver carbonate, saturated with hydrogen sulphide, filtered, evaporated to dryness, taken up with water, filtered and the filtrate evaporated to yield a glass (0.14 g.). This was treated with 2N-hydrochloric acid (1 c.c.) which was removed by continuous distillation with water to yield a syrupy mass. Filtration followed by heating at 100° gave a syrupy lactone. Yield after purification with acetone (0.026 g.) $[\alpha]_D$ -25° in water (c, 0.3).

Investigation of Fraction (3b)

Deacetylation and Lactone Formation.- The syrup (0.77 g.) was deacetylated as in the case of Fraction (1e) (page 108) to yield a brown mobile syrup (0.4 g.). This syrup was diluted with water (10 c.c.) and bromine (1 c.c.) added together with lead carbonate (5 g.) and the mixture allowed to stand 2 days. It was then worked up in exactly the same way as the lactone from fraction (1e)(see above), to yield a

glass (0.24 g.). This was treated as in the previous case to yield a syrupy lactone (0.18 g.).

Examination of the Lactone.- The lactone showed $[\alpha]_D^{15°}$ -17° (initial); -15° (4 days, constant), in water (c, 2.0).

Found: OMe, 7.5. Calc. for $C_7H_{12}O_6$, OMe, 16.1%. Titration showed the presence of about 50% of lactone in the equilibrium solution. 0.0222 g. required 2.40 c.c. N/40-sodium hydroxide to phenolphthalein at 15° and a further 2.40 c.c. on heating $C_7H_{12}O_6$ requires 4.6 c.c.

Weerman Reaction.- The rotation solution was evaporated and the syrupy product converted into the ester by boiling with methyl-alcoholic hydrogen chloride. Treatment with ammoniacal methyl alcohol yielded a syrupy amide ($[\alpha]_D^{15°}$ +8° (c, 0.7).

Investigation of Fraction (B)

Hydrolysis.- Various yields of Fraction (B) $[\alpha]_D^{18°}$ +14° (page 104) were combined (5 g.) and dissolved in N/2-oxalic acid (100 c.c.) and heated at 98-100° for 22 hours. An inorganic precipitate (0.63 g.) was deposited and removed by filtration. The filtrate was neutralised with calcium carbonate in the presence of charcoal, filtered and evaporation at 50°/16 mm. of the filtrate gave a syrup admixed with

solid (0.92 g.). Extraction with alcohol followed by removal of the latter gave a reducing syrup $[\alpha]_D$ +37° in water (c, 0.67) (OMe, 19.6%).

Acetylation.- The above syrup was acetylated as on page 99 to yield a brown syrup (4.3 g.). Distillation under high vacuum gave 3 fractions:

Fraction	Bath temp.	n_D	$[\alpha]_D$ $CHCl_3$	%OMe	Yield
1	120-160°/01mm.	1.4738	0° (c 0.7)		0.16 g.
2	160-180° "	1.4600	+19° "	16.6	3.45 g.
3	190-250° "	1.4622	+24° "	14.5	0.38 g.
			Residue		0.25 g.
					4.24 g.

Investigation of Fraction (2)

Deacetylation and Osazone Formation.- Fraction (2) (0.49 g.) was deacetylated according to Zemplen's method as on page 108, and the product converted into the osazone. Various yields of osazone were obtained the m.p. of which varied from 175-190° and methoxyl content from 7.4-8%. Repetition of this on fresh specimens of fraction (2) gave light yellow crystals of osazone whose m.p. varied from 175° in the case of one yield to 184° in another.

Changes in Rotation of Deacetylated Fraction (2) during Glycoside Formation: Deacetylated fraction (2) (0.095 g.) was dissolved in dry methyl alcohol (8 c.c.)

and gave $[\alpha]_D^{17°}$ +43°, sufficient dry methyl-alcoholic hydrogen chloride was added to bring the concentration of hydrogen chloride in the solution up to 1% (allowance being made for the presence of sodium) $[\alpha]_D$ +38.5°(initial); +10°(2 hours); +5° (4.5 hours); +3° (45 hours); -12° (70 hours); -14° (95 hours, constant).

Changes in Rotation of Galactose during Glycoside Formation.- $[\alpha]_D^{16°}$ in 1%methyl-alcoholic hydrogen chloride (c, 1.5) +61° (initial); +34° (8 hours); -10° (27 hours); -42° (72 hours); -49° (100 hours, constant).

Complete Methylation of Deacetylated Fraction (2).- The deacetylated syrup (0.17 g.) was subjected to two Purdie methylations, as on page 100, to yield a pale yellow syrup. The glycosidic methoxyl residue was removed in the usual way to yield a pale yellow mobile syrup. Nucleation with a crystal of tetramethyl glucose failed to yield crystals.

Anilide Formation.- Anilide formation, (page 100), followed by nucleation with a crystal of tetramethyl galactose anilide gave crystalline tetramethyl galactose anilide (0.016 g.), m.p. 195°. Mixed m.p. with an authentic specimen showed no depression.

Nitric Acid Oxidation of Deacetylated Fraction (2).- A further quantity of Fraction (2) was deacetylated to give a clear brown syrup (0.4 g.). This was

oxidised with nitric acid (3 c.c. concentrated) at 70° for 6 hours, and then freed from acid by repeated additions of water and distillation. The residue was boiled with 4% methyl-alcoholic hydrogen chloride for 8 hours, neutralised with silver carbonate, filtered, taken to dryness, extracted and filtered to yield a syrup (0.21 g.). Distillation in a high vacuum yielded a very small quantity of syrup which on treatment with methyl-alcoholic ammonia gave a few needles on standing. Treatment of the residue in the distillation flask with methyl-alcoholic ammonia gave no results.

Course of the Hydrolysis of the C.E. with N-Sodium Hydroxide

Dried C.E. (1.771 g.) was treated with N-sodium hydroxide (200 c.c.) at 100° and the quantity of sulphate liberated was estimated gravimetrically (30) as barium sulphate, at definite intervals, by filtration through a sintered crucible. A Jena flask was used which had been shown to yield no silica when heated for similar periods with N-sodium hydroxide.

% Hydrolysis calculated on a total yield of 35.1% SO_4

10 c.c. after 1 hour	gave	0.009 g. $BaSO_4$	= 14.5%	hydrolysis
10 c.c. " 4.7 "	"	0.0249 g."	= 40.2%	"
10 c.c. " 8.16"	"	0.0312 g."	= 50.3%	"
10 c.c. " 13 "	"	0.0356 g."	= 57.4%	"
10 c.c. " 23 "	"	0.0492 g."	= 79.3%	"
10 c.c. " 31.75"	"	0.0559 g."	= 90.0%	"
10 c.c. " 51.5 "	"	0.0608 g.	= 98.0%	" const.

Comparison with Potassium β-Methylgalactoside Sulphate under Similar Treatment

Barium β-methylgalactoside sulphate (1.2026 g.) was treated with the appropriate quantity of potassium sulphate (0.2863 g.) and the insoluble barium sulphate removed by filtration. The potassium β-methylgalactoside was then treated with 4% sodium hydroxide (100 c.c.) and the sulphate estimated as before (30).

10 c.c. after 0.5 hours gave 0.0273 g. $BaSO_4$ ie. 41.8% SO_4

10 c.c. " 2.3 " " 0.0655 g. " ie. 100% SO_4.

Investigation of the Product after Hydrolysis of C.E. with N-Sodium Hydroxide

C.E. (27 g.) was treated with N-sodium hydroxide (1350 c.c.) on a constant level bath at 90° for 60 hours. The product was dialysed for 10 days and on evaporation gave a brown transparent solid (10 g.). Ash 5% (mainly silica). Calcium, nil.

Hydrolysis with N/2-Oxalic Acid

The above product (2 g.) was hydrolysed with N/2-oxalic acid (50 c.c.) on a water-bath at 90° for 30 hours. The product was worked up in the usual manner to give a syrup (1.70 g.), $[\alpha]_D$ +33° (c, 0.07), which gave 0.760 g. galactosephenylmethylhydrazone corresponding to 49.4% galactose in the syrup.

S_U_M_M_A_R_Y

1. The isolation of a polysaccharide from carrageen moss by extraction with cold water is described. The metallic constituents of the ash are shown to be: sulphate, 63.8%, calcium, 5.5%, potassium, 24.5%, sodium 13.7%, and the fact that the ratio of sulphate obtained on hydrolysis to the sulphate contained in the ash was approximately 2:1 indicated the substance to be an ethereal sulphate. The proportion of d-galactose obtained on hydrolysis was shown to be 34%.

2. No evidence for the presence of glucose in the products of hydrolysis was found although it was shown that β-methylglucoside tetraacetate could be isolated from the H.E. It is sho wn that the hydrolysis of a simple ethereal sulphate by acid does not proceed with the formation of an anhydro-ring in the case of barium β-methylgalactoside sulphate.

3. Methylation yielded a product containing OMe, 14.5%, from the hydrolysis products of which crystalline 2-methyl β-methylgalactoside was isolated. 6-Methyl β-methylgalactoside was prepared for the first time for comparison. Galact-

osazone was obtained from the hydrolysis products of methylated C.E. which agrees with the view as to the presence of 2-methyl galactose. Complete methylation of the hydrolysis products gave tetramethyl galactopyranose anilide.

The same 2-methyl galactose derivative was isolated from the hydrolysis products of methylated H.E. thus confirming a previous view that 2-methyl galactose was present in this extract also.

4. Treatment of C.E. with .013N-sulphuric acid having given a product with a slightly higher galactose content than the original, the same treatment was applied to the methylated C.E. This gave two fractions: (A) which was precipitated by alcohol from aqueous solution, and (B) which remained in solution.

5. By the hydrolysis and fractionation of (A) two fractions were obtained both of which gave tetramethyl galactopyranose anilide on suitable treatment.

Fraction (1) gave 6-methyl galactosazone and a γ-lactone of negative rotation indicating the absence of a methoxyl group on C_4 and since free 6-methyl-galactose appeared to be absent the dimethyl gal-

actose is considered to be 2:6-dimethyl galactose. Fraction (2) gave galactosazone and a γ-lactone, the corresponding amide of which gave a negative Weerman test. This fraction is therefore concluded to be chiefly 2-methyl galactose.

6. The more soluble fraction (B), obtained in variable yield, on hydrolysis, gave a mixture of sugars of slightly higher methoxyl content than the corresponding products from (A). Evidence from experiments on glycoside formation indicates that position 4 carries a free hydroxyl group, and one methoxyl residue is lost on treatment with phenylhydrazine. The chief component is concluded to be 2:6-dimethyl galactose although difficulties in purification prevented the precise identification of the monomethyl galactosazone isolated. From the foregoing facts it is concluded that the polysaccharide sulphate carries free hydroxyl groups on C_2 and C_6.

7. Measurements were carried out on the rate of hydrolysis of the sulphate residue with alkali, and, in agreement with the results of Haas, the reaction is shown to be slow. Comparison with potassium β-methylgalactoside sulphate under the same conditions leads to the conclusion that position 3 cannot

carry the sulphate group if C_6 is free (or *vice-versa*) since rapid hydrolysis would be expected in this case. The sulphate group is therefore assigned to position 4, and the linkage between the units to 1 (since the substance is non-reducing) and 3 as in agar.

8. This view is supported by the proof that the proportion of galactose liberated from the C.E. after treatment with alkali followed by hydrolysis with acid is not lower but slightly higher than the sugar produced by direct hydrolysis.

B I B L I O G R A P H Y

1. Flückiger and Obermayer ----Report Pharm.,1868, 380.
2. Bente ----------Ber., 1876, 9, 1157.
3. Haedicke, Bauer, and Tollens Annalen, 1887,238, 302.
4. Müther and Tollens -------- Ber., 1904, 37, 302.
5. Lintner, Düll and Kiermayer -- Ber., 1895, 28, 243.
6. Tollens ---------- ------- Handbuch der Kohlenhydraten, 1914, 3rd. Edition.
7. Sebor ---------- Oesterr. Chem. Zeit.,1900,3,441
8. Haas and Hill ---------- Ann. Appl. Biol.,1921,7,352.
9. Haas ---------- Biochem.J., 1921,15, 469.
10. Russell-Wells -------- ---- Biochem.J., 1922, 16, 578.
11. Harwood ---------- J., 1923,123, 2254.
12. Haas and Russell-Wells ---- Biochem.J.,1929, 23, 425.
13. Forbes and E.G.V.Percival - J., 1939, 1844.
14. Butler ---------- Biochem.J.,1934,28,759.
15. Nelson and Cretcher ------- J.Biol.Chem.,1931,94,147.
16. Dillon and O'Colla -------- Nature, 1940, 145, 749.
17. Buchanan and E.G.V.Percival - Nature, 1940, 145, 1020.
18. Bredereck ---------- Ber., 1931, 64, 1730.
19. Hassid ---------- J.A.C.S., 1933, 55, 4164; 1935, 57, 1048.
20. E.G.V.Percival and Somerville- J., 1937,(2),1724.
21. Hirst and Jones ---------- J., 1939, 1482.
22. Smith ---------- J., 1939, 1724.
23. Oldham and Bell ---------- J.A.C.S., 1938, I, 324.
24. Freudenberg and Smeykal --- Ber., 1926, 59, 100.
25. Robertson and Lamb -------- J., 1934, 1321.

26. E.E.Percival and E.G.V.Percival - J., 1938, 1587.

27. E.G.V.Percival and T. Soutar ---- J., 1940, 1475.

28. E.G.V.Percival and R.B.Duff ----- J., 1941, 830.

29. Haworth,Owen and Smith ---------- J., 1941, 88.

30. Cumming and Kay ---------- Qualitative Chemical Analysis, 7th.Ed.225.

31. Ibid ---------- " page 107

32. Ibid ---------- " " 326.

33. Ibid ---------- " " 335.

34. Meyer ---------- Analyse und Konstitutions-ermittlung organischer Verbindungen,5 Auflage,p.483.

35. Lüdtke ---------- Biochem.Z.,1929,419.

36. Baldwin and Bell ---------- J., 1938, 1465.

The author would like to render thanks to her husband for his direction and advice during the course of this work.